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REGIONAL AND SEASONAL DISTRIBUTION OF MOISTURE, CARBOHYDRATES, NITROGEN AND ASH IN 2-3 YEAR PORTIONS OF APPLE TWIGS

A THESIS
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA

BY

HAMILTON PAUL TRAUB

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
JUNE 1927
MINNEAPOLIS

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REGIONAL AND SEASONAL DISTRIBUTION OF MOISTURE, CARBOHYDRATES, NITROGEN, AND ASH IN 2-3 YEAR PORTIONS OF APPLE TWIGS[1]

By Hamilton Paul Traub

INTRODUCTION

An understanding of the physiology of woody plants is of first importance to those engaged in the culture of trees and shrubs. Only by a clear conception of the functioning of the tissues of the woody plant can any real progress be made toward the solution of such important problems as translocation of food, fruit-bud formation, and winter hardiness in trees. Anything that might add to the accumulating scientific knowledge of the physiology of the apple tree would serve as part of the foundation upon which the superstructure of cultural practices may be based. The experiments reported in this paper were undertaken in September, 1924, in the hope of making a slight contribution toward a clearer understanding of the functioning of the complex tissues of the apple tree. The main interest centers around a purely physiological problem; but any advance in the basic sciences usually finds immediate application in the related applied sciences.

In carrying out the research, the method of macrochemical analysis of the complex tissues of the 2-3 year portions of apple twigs was utilized in an attempt (1) to extend the researches in tree physiology initiated by Geleznow (1878), at St. Petersburg (Leningrad); and Craib (1918), at Edinburgh, on moisture relations; Alfred Fischer (1888, 1891), and Niklewski (1906), at Leipzig, on the starch-oil and starch-sugar transformations in woody plants, and (2) to study the distribution of nitrogen and mineral matter in the apple tree on the basis of complex tissues throughout the season.

The work is developed under the following heads: (1) Materials and methods, (2) variation in the relative proportions of the complex tissues, (3) regional and seasonal distribution of moisture, (4) regional and seasonal distribution of carbohydrates, (5) regional and seasonal distribution of nitrogen, (6) regional and seasonal distribution of ash, (7) practical application, (8) conclusions, and (9) summary.

[1] Also published as Minn. Agr. Expt. Sta. Tech. Bull. 53. 1928.

MATERIALS AND METHODS

This section is devoted to a more detailed description of the materials and methods employed in this research, which were briefly described by Traub (1927).

In order that results from one date to another should be comparable, special care was taken to obtain sufficient homogeneous material to carry through the experiment for at least two years. After considering other possibilities, including a plan of cutting down one 11-year-old apple tree for each sample, it was finally decided to limit the study to the 2-3 year portion of apple twigs. According to this plan, portions (of the dormant twig) in the second year of growth, in March, were taken as the first material for analysis. Similar portions of twigs were taken for analysis on selected dates from March (into the third year of growth) to February of the following year. With the adoption of this

Fig. 1. Two 11-year-o'd "University" apple trees at the beginning of the experiment, September, 1924. Trees planted at University Farm, St. Paul, Minnesota, May 6, 1914.

method it was possible to confine operations to a single or at most two trees of the same variety growing under similar conditions and with similar performance records, for the amount of material removed in any one year was not sufficient to change unduly the nutritive balance of the trees. This procedure made possible an intensive study of the complex tissues of one winter-hardy apple variety under the climatic conditions of central Minnesota. The results obtained should, therefore, be interpreted on this basis.

Two 11-year-old "University" apple trees *(Pyrus malus)* were used for the experiment. This is a late fall, yellow-fruited seedling of Oldenburg originated and introduced by C. G. Patten, Charles City, Iowa, in 1882. The variety has been described by Hansen (1902) and Green (1903). The tree is an annual bearer; vigorous and quite winter hardy under central Minnesota conditions. The trees selected are located on the Agricultural campus, University of Minnesota, St. Paul. They were planted in May, 1914, in silt loam with a clay subsoil, and were in sod culture after the first few years of clean cultivation. The trees have always been vigorous and have similar performance records. Since they came into bearing, the trees have produced comparatively light crops of fruit annually. The condition of the trees at the beginning of the experiment, September, 1924, is portrayed in Figure 1. The

Fig. 2. The two "University" apple trees toward the end of the experiment, Dec. 11, 1926. Trunk diameter 6½ and 7 inches, height 16½ and 16¾ feet, and spread 16 and 17 feet.

same trees toward the end of the experiment, December, 1926, are shown in Figure 2. At this time the trunk diameter of the trees measured 6½ and 7 inches respectively; the height 16½ and 16¾ feet; and the spread 16 and 17 feet.

Collection and Preparation of Materials

Samples were taken at twenty different times during the season, but not at regular intervals. During the periods April to May and September to October, when environmental changes bring about very pronounced physiological transformations, studies were more intensive than at other seasons of the year. All the samples were analyzed for total moisture and reducing and total sugars. With these facts as a basis, what appeared to be critical periods in the seasonal cycle were selected. The

remaining material collected on these dates was used for additional determinations: acid-hydrolyzable alcohol-insoluble carbohydrates, pentosans, total and amino nitrogen, and ash.

About 125 grams of material were collected for each sample, always between the hours of 7:30 and 8:30 in the morning, within a range of several feet and about 8 feet from the ground from the north, south, east, and west sides of the trees. The material was brought to the laboratory in a vasculum and was quickly separated by means of a sharp grafting knife into the four fractions—periderm,[2] cortex-phloem, outer xylem, and inner xylem-pith, as indicated in Figure 3. A longitudinal incision was made in the twig with a sharp grafting knife, and the periderm was then peeled off as indicated in Figure 3. During most seasons of the year the periderm was fairly easily separated, but this operation was usually the most tedious part of the procedure as great care had to be exercised not to take parts of the cortex. The tissues between the periderm and xylem tissues, designated hereafter as cortex-phloem, were next scraped off as a second complex tissue, leaving the wood and the pith. The outer greenish-tinged xylem was stripped off as a third complex tissue by means of a sharp grafting knife, leaving the inner whitish and drier xylem-pith as a fourth complex tissue. The various complex tissues were kept in closed specimen jars after separation. The separated material in each case was cut into small pieces by means of pruning shears and the pieces were thoroly mixed. The fractions of each of the four complex tissues for the macrochemical analyses were then preserved.

Preservation of Material

For the quantitative macrochemical analyses the material representing each of the four complex tissues—periderm, cortex-phloem, outer xylem, and inner xylem-pith—was divided into two major fractions:

(a) Fractions for drying to be used for the determination of moisture, organic nitrogen, and ash.

(b) Fractions to be preserved in alcohol for the determination of reducing and total sugars, acid-hydrolyzable alcohol-insoluble carbohydrates, pentosans, and amino nitrogen.

The first major fractions were dried to constant weight at 102°C. for moisture determinations, and the residue was then ground in a drug mill to a fine powder to be used for the estimation of organic nitrogen and ash.

The second major fractions of the cortex-phloem, outer xylem, and inner xylem-pith were again divided into duplicate samples for analysis.

[2] Eames, A. J. and MacDaniels, L. H. An introduction to plant anatomy. First ed., p. 213. New York. 1925. Confirmed by letter from A. J. Eames, Cornell Univ., Ithaca, N. Y., Dec. 8, 1926.

Because of the small quantity of periderm tissue, single samples were preserved.

The material was preserved by putting it immediately after the final mixing and weighing into Mason jars containing boiling 95 per cent alcohol to which 1 gram of calcium carbonate had been added. After boiling for 30 minutes, the jars were sealed and stored for analysis at a convenient time.

Methods of Analysis

The samples were extracted with alcohol in Landsiedl extractors over a steam bath for 24 hours. Previous experiments had shown that after 18 hours any further extraction was equivalent to a blank, even when the tissue was broken further.

The alcohol-insoluble residue remaining in the extraction cup was then dried to constant weight at 102°C. and ground in a drug mill to a fine powder to be used in the determination of total acid-hydrolyzable

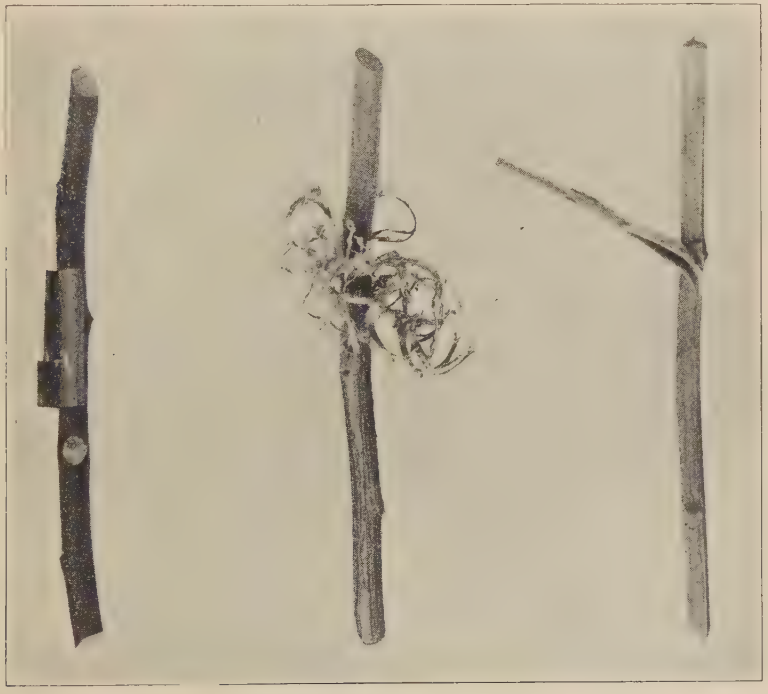

Fig. 3. Method of separating complex tissues—periderm, cortex-phloem, outer xylem, and inner xylem-pith.

alcohol-insoluble carbohydrates (hexosans and pentosans), and pentosans. After making up to volume, the alcohol-soluble extract was treated with chloroform to separate the crude lipides (fats, lecithin, chlorophyll, etc.). The remaining alcohol-soluble phase was divided into

aliquots for the determination of reducing sugars, total sugars, and amino nitrogen, the alcohol being removed in Claissen flasks under reduced pressure at a temperature of 40-60°C.

Duplicate samples were analyzed, except in the case of the periderm material, and all analyses were made in duplicate except those of the sugars, which were in triplicate.

The official methods of analysis of the Association of Official Agricultural Chemists (1925) were used throughout with a single slight modification as indicated under carbohydrate analyses.

Carbohydrate Analyses

In the determination of reducing and invert sugars according to the Munson and Walker method, the volumetric thiosulphate method of estimating precipitated cuprous oxide was used except that bromine water was omitted from the process.

In the estimation of total sugars, hydrolysis was accomplished by allowing 50 cc. of the clarified solution, to which 10 cc. of dilute hydrochloric acid (sp. gr. 1.1029) had been added, to stand 24 hours at a temperature of 20-24°C.

According to Harvey (1923, 1925), the presence of phloridzin in apple tissue may lead to serious errors in sugar determinations unless necessary precautions are taken in technical procedure. Harvey suggested, however, that the method of inversion at room temperature for 24 hours might avoid the hydrolysis of phloridzin. This was essentially the method of hydrolysis employed in all the sugar determinations, but it was considered advisable to check this point. For this purpose phloridzin was tested in saturated solution before and after hydrolysis at room temperature for 24 hours, by using 25-cc. portions for reduction with standard Fehlings solution. The results were equivalent to blank determinations in each case.

The method of direct acid hydrolysis was used in the determination of the acid-hydrolyzable alcohol-insoluble carbohydrates, i.e., acid-hydrolyzable material including starch. The results obtained by this method include the pentosans and other carbohydrate bodies that undergo hydrolysis and are converted into reducing sugars on boiling with hydrochloric acid. Two grams of dry material was stirred in a beaker with 50 cc. of cold water for an hour, transferred to a filter, and washed with 250 cc. of cold water. The insoluble residue was heated for 2½ hours with 200 cc. of water and 20 cc. of dilute hydrochloric acid (sp. gr. 1.125) in a flask provided with a reflux condenser. After cooling, nearly neutralizing with sodium hydroxide, completing volume to 250 cc., and filtering, the glucose was determined in aliquots of the filtrate.

Pentosans were determined by the phloroglucinol method. The quantity obtained was subtracted from that of the acid-hydrolyzable

alcohol-insoluble carbohydrates, leaving the acid-hydrolyzable hexosans, including starch.

Nitrogen Analyses

Amino nitrogen was determined by the Van Slyke method. The mixture of glacial acetic acid, sodium nitrite, and the solution of the sample was shaken for 5 minutes.

The total organic nitrogen was determined according to the Gunning modification of the Kjeldahl method.

Ash Analyses

Total ash was determined by burning in a porcelain crucible 2 grams of the dry powdered residue from the moisture determinations for each analysis in a muffle oven until a white or grayish white residue was obtained.

Tables and Graphs

The complete analytical data are given in Tables I, II, and IV to XV inclusive. The results are shown graphically in Figures 4 to 20 inclusive.

VARIATION IN RELATIVE PROPORTIONS OF COMPLEX TISSUES

That the relative proportions of the complex tissues did not remain constant from one date to another under the conditions of the research, was determined in the preliminary experiments from September to December, 1924. This fact was also reported by Proebsting (1925), but he made no attempt to put it on a quantitative basis.

From a theoretical viewpoint, the difference in the relative proportions of the complex tissues at various seasons of the year may be due to (1) variation in the diameter of the twigs utilized, introducing varying volumes of the complex tissues, (2) variation in the dry weight without change in volume due to the translocation of substances into and out of a region, and (3) growth (increase in tissue) or senescence (decrease in tissue due to absorption after death) in a part of the complex tissues without a corresponding change in the remaining tissues. Further experimentation is necessary to establish the relative importance of these various sources of error when entire twigs are analyzed at various seasons of the year.

Errors due to variation in diameter of the twig can be minimized by selecting twigs as nearly alike in diameter as possible throughout the experiment. There seems to be no remedy for errors due to the normal seasonal physiological processes of the plant—translocation of material into and out of a region, and growth or senescence of tissues.

The seasonal variation in the relative proportions of the complex tissues throughout the experiment are indicated in Table I in per cent dry weight, and are shown graphically in Figure 4.

TABLE I

SEASONAL VARIATION IN RELATIVE PROPORTIONS OF PERIDERM, CORTEX-PHLOEM, OUTER XYLEM, AND INNER XYLEM-PITH, IN 2-3 YEAR PORTIONS OF APPLE TWIGS, MARCH 7, 1925, TO FEBRUARY 26, 1926

Expressed in per Cent of Total Dry Weight

Date	Periderm	Cortex-phloem	Outer xylem	Inner xylem-pith	Periderm plus cortex-phloem	Outer xylem plus inner xylem pith
March 7	8.25	17.21	44.31	30.23	25.46	74.54
April 4	8.10	18.97	43.05	29.88	27.07	72.93
May 16	8.16	19.85	44.24	27.75	28.01	71.99
June 20	8.61	15.02	44.99	31.38	23.62	76.38
September 17..	4.37	17.75	44.26	33.62	22.12	77.88
October 10....	8.06	18.22	38.93	34.79	26.28	73.72
October 23....	3.10	16.68	46.98	33.24	19.78	80.22
December 23 ..	6.17	17.73	41.46	34.64	23.90	76.10
February 26 ..	7.66	18.78	41.56	32.00	26.44	73.56
Average ..	6.94	17.80	43.31	31.95	24.742	75.258

The data given in Table I show that on the basis of complex tissues—periderm, cortex-phloem, and outer xylem plus inner xylem-pith[3]—there are marked fluctuations in the relative proportions of these complex tissues. The variations are quite significant, amounting to 8.23 per cent on a dry weight basis in the periderm plus cortex-phloem and outer xylem plus inner xylem-pith.

Tables IV, V, and VIII show that in the spring the xylem tissues, composing over 70 per cent of the total dry weight, and functioning as winter storage organs, lose a relatively large part of their stored material by translocation, and the relative proportion of the cortex-phloem rises correspondingly. In late October this is reversed—a relatively large part of the reserve food is translocated and stored in the xylem tissues, and there is a corresponding decline in the relative proportions of periderm and cortex-phloem. Other factors may, however, be operative. Hanson (1926) has shown that in *Acer saccharinum* under eastern Nebraska conditions, xylem formation begins between April 20 and 27, when leaves are about half grown; production of new cells is very active throughout May and June, and by August 1 most of the xylem is formed and over 90 per cent is lignified. By August 15 lignification is complete to the cambium, and no new cells are being formed; by September 14 cambial activity is renewed, and new xylem is being developed, and by October 14 cell formation has ceased and the xylem is completely lignified. The abrupt variations in the relative proportions of the complex tissues in 2-3 year portions of apple twigs

[3] The outer xylem and inner xylem-pith are not considered separately, as it may be argued that an error might be introduced when separations are made.

during April-May, and September-October, under central Minnesota conditions, may be connected with cambial activity. There is also the possibility that during periods of cambial activity, undifferentiated cells on the xylem side of the cambium may adhere to the cortex-phloem

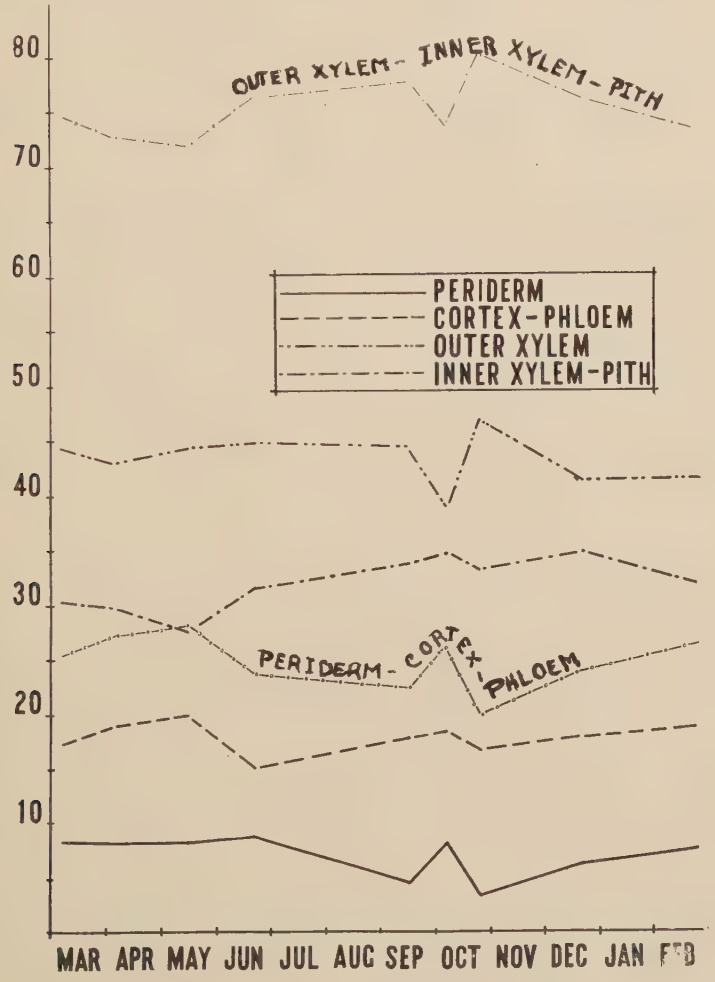

Fig. 4. Seasonal variation in the relative proportion of periderm, cortex-phloem, outer xylem, inner xylem-pith, periderm plus cortex-phloem, and outer xylem plus inner xylem-pith. Expressed in per cent dry weight.

fraction during the separation, thus apparently increasing the relative portion of this tissue. However, the proportion of the phloem begins to rise in early March when the trees are dormant and before there is likely to be any cambial activity, indicating that the change is due apparently to translocation of material from the xylem tissues.

In conclusion, the gross analysis of the plant organs (twigs, spurs, roots, etc.) in seasonal studies is on the defensive.[4] At least, the error that may be introduced by using this method should be fully recognized in the interpretation of data based upon the analysis of entire plant organs composed of complex tissues that may vary in their relative proportions from one date to another. The whole problem requires thoro investigation.

REGIONAL AND SEASONAL DISTRIBUTION OF MOISTURE

In the study here reported, an attempt was made to come a little closer to a quantitative estimation of the seasonal moisture relations of the complex tissues of 2-3 year portions of apple twigs—periderm, cortex-phloem, outer xylem, and inner xylem-pith. We are not concerned alone with such seasonal variation of total moisture content of the stem as reported, for instance, by du Sablon (1904, 1906) for the pear and other trees, but rather with an estimation of the individuality of the various complex tissues with respect to seasonal moisture distribution and its physiological significance.

Previous Work

Several workers have reported experiments on the subject of seasonal and regional variation of the moisture content in the stems of woody plants.

Geleznow (1876), at St. Petersburg (Leningrad), carried out experiments showing the seasonal distribution of moisture in the cortex-phloem and wood of *Acer platanoides, Betula alba, Populus tremula,* and *Pinus sylvestris.* He concluded that the cortex-phloem usually contains more moisture than the wood.

Craib (1918, 1920, 1923) reported experiments on the regional spread of moisture throughout the year in the trunk of *Acer pseudo-platanus,* and other trees. He showed that there are considerable seasonal fluctuations of moisture in the different portions of the wood: at the beginning of the leafless period, October, the center of the tree is very wet; at the end of the same period, March, there is a very wet region near the outside and the center is very dry; between these two extremes occur all the intermediate stages. During the leaf-bearing period, March to May, it appears probable that the moisture goes through the same movement on a reduced scale and at a greatly accelerated speed as in the longer interval, October to March. When the

[4] It may be argued that the same criticism that applies to the analysis of the plant organ made up of complex tissues would also apply, to a lesser extent, to the comparative study of complex tissues. This is true, but for practical reasons it will probably not be possible to separate the complex tissues into simple tissues for macrochemical analysis.

full-leaf stage is reached, throughout most of the tree the whole central area of the wood has an almost uniform percentage of moisture, and the youngest wood has a relatively high moisture content.

Immink (1923) reported experiments with teakwood and mahogany trees in which he claims to have obtained results similar to those reported by Craib with *Acer pseudoplatanus*.

Craib (1925)[5] suggested to the writer that Craib's results (1918) might be connected with the work of Templeton (1923) on starch distribution in the trunk of *Acer pseudoplatanus* at various heights above ground level. Templeton used the same series of trees and followed the same plan for the different level cuts and his results should, therefore, be comparable with those of Craib (1918).

General Tendencies

The researches reported above were concerned either with cortex-phloem and xylem or with xylem tissues alone. In this work the seasonal distribution of moisture has been followed in the four complex tissues—periderm, cortex-phloem, outer xylem, and inner xylem-pith. The results obtained are expressed as moisture in per cent dry weight in Table II, and are shown graphically in Figure 5.

TABLE II

REGIONAL AND SEASONAL DISTRIBUTION OF MOISTURE IN 2-3 YEAR PORTIONS OF APPLE TWIGS, MARCH 7, 1925, TO FEBRUARY 26, 1926

Expressed in per Cent Dry Weight

Date	Periderm	Cortex-phloem	Outer xylem	Inner xylem-pith	Whole twig
March 7	17.13	96.04	58.57	45.93	57.77
March 27	28.79	97.17	47.08	47.80	54.78
April 4	58.33	65.33	53.83	53.23	55.31
April 18	65.62	117.19	61.16	57.92	71.05
May 2	75.12	153.37	72.56	66.24	86.53
May 16	80.98	125.88	70.76	71.65	82.78
May 30	81.69	139.20	80.21	73.50	90.20
June 20	35.01	167.16	92.03	71.32	91.89
July 18	44.17	120.79	91.94	71.65	87.41
August 18	57.25	132.68	65.01	66.88	74.70
September 17	67.61	119.64	79.76	64.49	81.17
October 4	52.46	102.89	74.27	52.61	70.45
October 10	48.65	100.83	58.51	52.44	60.89
October 23	64.63	106.75	58.77	59.39	67.16
December 23	29.52	74.85	61.59	47.40	57.04
January 29	24.11	62.74	59.00	55.14	56.73
February 26	18.15	88.96	54.43	56.26	58.72

The results show wide variation in amount of moisture for the various tissues. During certain times of the year there is also a difference in trend in the periderm.

[5] Letter from William G. Craib, Dept. of Botany, Univ. of Aberdeen, Old Aberdeen, Scotland, Dec. 6, 1925, to Hamilton P. Traub.

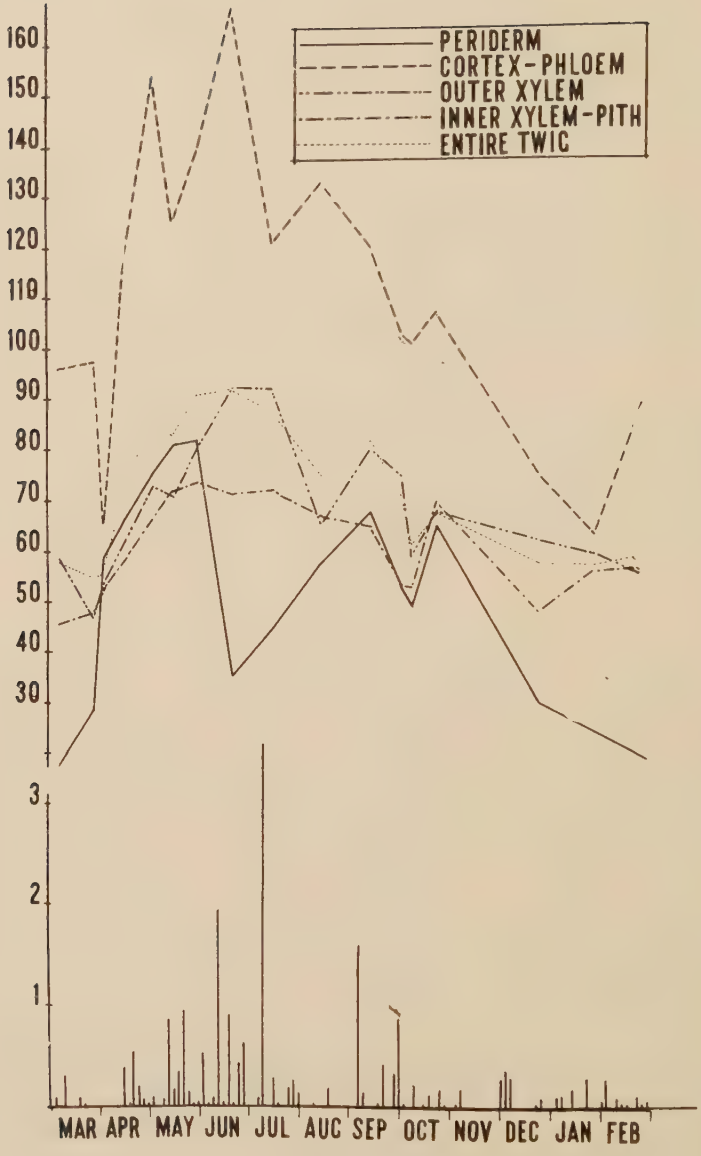

Fig. 5. Upper, seasonal distribution of moisture in periderm, cortex-phloem, outer xylem, inner xylem-pith, and entire twig. Expressed in per cent dry weight.

Lower, precipitation from March 1, 1925, to February 28, 1926, in three-day summation periods. Expressed in inches. From Annual Meteorological Summary, 1925 and 1926, U. S. Weather Bureau, Minneapolis, Minnesota, Station.

The highest percentage of moisture during the entire year is found in the cortex-phloem. Geleznow (1876) found a similar condition in *Acer* and other trees. The range in the cortex-phloem on a dry weight basis varies from 65.33 to 167.16 per cent, a difference of 101.83 per cent. The cortex-phloem contains a relatively greater proportion of living tissue than the xylem and a correspondingly greater moisture content would be expected. This is actually the case.

The two xylem tissues, outer xylem and inner xylem-pith, follow the same trend except that the per cent moisture in the outer xylem shows a tendency to run higher at practically all times of the year. This is especially marked during the summer. There is a range from 47.08 to 92.03 per cent, a difference of 44.95 per cent in the outer xylem; and a range from 47.80 to 73.50 per cent, a difference of 25.70 per cent, in the inner xylem-pith. There is, therefore, a significant and consistent lower moisture content in the xylem tissues than in the cortex-phloem.

The moisture content of the periderm is usually lower than that of the xylem tissues. There is a comparatively wide range, from 17.13 to 81.98 per cent, a difference of 64.85 per cent, but the absolute amount reaches above that of the xylem tissues for relatively short periods only. In the periderm, there is not only a difference in amount but also a difference in trend from the end of May to the middle of August. While the moisture in the cortex-phloem and outer xylem rises from the end of May to the end of June, the moisture in the periderm falls off; when the moisture falls in the cortex-phloem, from the end of June to the middle of July, and in the outer xylem from the end of June to the middle of August, the moisture rises in the periderm. After the middle of August the moisture content of the periderm has the same trend as in the other tissues, but it has a tendency to be lower than in the other tissues during the winter, with a temporary rise above the xylem tissues from early April until the end of May.

When the moisture data are calculated on the basis of the entire twig, the individuality of the complex tissues naturally disappears and the tendency of the tissue, outer xylem, in greatest relative abundance, is reflected. The range of moisture content, 54.78 to 91.89 per cent, a difference of 37.11 per cent, closely approaches that of the outer xylem. The error introduced by this method of expressing quantitative results has been emphasized above.

In the following discussion the moisture relations in the four complex tissues will be followed through the physiological periods—the growing period, the transition between growing and dormant periods, the dormant period, and the transition between dormant and growing periods.

Growing Period—April to September

In general, the summer maximum of moisture in all the complex tissues is reached, under central Minnesota conditions, between April 3 and September 14, when precipitation is greatest—over 70 per cent of the annual total.

The fluctuations in amount of moisture during the season are considerable in these four tissues. The rise in early April begins before much precipitation has fallen. The rains begin about the middle of April but the first maximum is not reached by all the tissues until May. There is a temporary decline in moisture content of the cortex-phloem and outer xylem, followed by another maximum in June for the cortex-phloem and from late June to the middle of July for the outer xylem. The summer minimum of the periderm is attained at this time. It seems strange that there should be an actual decline in moisture in the cortex-phloem during the month of heaviest rainfall, July, and an increase to a third maximum during the month of least rainfall, August. The decline of moisture content in periderm and cortex-phloem during periods of abundant rainfall points to the fact that when there is an optimum water supply the actual amount of water taken up is limited by vital phenomena and that under such conditions there is no direct proportionality between total rainfall and the amount of moisture in the individual complex tissues. The correlation between the total moisture in the twig and total rainfall, however, is much closer, following the trend of the outer xylem, which as an individual tissue shows the same relationship. The outer xylem apparently serves as a complex water-supplying tissue. The periderm and cortex-phloem seem to act independently of the total precipitation within the summer maximum, selecting varying amounts of water from the common supplying tissue with the changing chemical and physical state of the protoplasm.

The inner xylem-pith reaches a maximum moisture content by the middle of May and shows only very slight fluctuations, and a very gradual downward trend to the middle of September. The rapid decline in moisture content of the periderm may be explained by the fact that the phellem tissue once formed soon becomes suberized and ceases to function as living tissue. Von Mohl (1847) demonstrated that dead plant tissue loses moisture more rapidly than living tissue. Accordingly, the moisture content declines rapidly after the end of May to the latter part of June. In this connection it is of interest to note the graphs, Figures 14 and 15, representing the nitrogen changes during this period. Figure 14 shows that during the time when the periderm tissue is being built up, April and May, the non-amino, or protein, content is relatively high, 71.4 to 73.34 per cent of the total nitrogen; and Figure 15 indicates that the amino acids decline rapidly

during the same period, 0.296 to 0.168 per cent on a dry weight basis. Altho further experimentation is necessary to decide this point, it appears possible that with the maturity, specialization, and differentiation of the periderm tissue, the non-amino nitrogen decomposes into the decomposition amino form. The latter undergoes a rise from 26.66 to 45.40 per cent of total nitrogen, and 0.168 to 0.286 per cent on a dry weight basis. This is shown in Figures 14 and 15.

In general, the periderm attains the first summer moisture maximum at the end of May and a second high peak is noticed in the middle of September before the autumnal yellowing of leaves sets in. During the first part of the period of heaviest rainfall, there is a sharp decline in the moisture of the periderm tissue. The cortex-phloem is most subject to fluctuation within the summer maximum, the outer xylem is less variable, and the inner xylem-pith maintains a fairly constant maximum.

Transition Between Growing and Dormant Periods

The most striking phenomenon during the transition period, September and October, is the sudden temporary decline in percentage of moisture content in all the complex tissues after the middle of September. The falling off in moisture begins while the leaves are still green, after the middle of September. When the fall minimum is reached, about the middle of October, the leaves are still green. The leaf blades were practically brown, and the petioles were colored red with anthocyanin by October 23, when a new moisture maximum was reached by all the complex tissues. When the fall maximum has been reached, growth has practically ceased and storage has been completed. At this time the moisture content of the inner xylem-pith is slightly greater than that of the outer xylem. Craib (1918) made similar observations for *Acer pseudoplatanus* later in the season. No direct evidence is offered to explain the temporary decline in moisture content during this period. The change is undoubtedly bound up with the chemical and physical changes that take place with the seasonal transformations. The drop takes place in spite of sufficient rainfall, as indicated in Figure 5. Further experimentation is necessary to clear up this matter.

Dormant Season—October to April

Various workers have reported experiments that have a bearing on the variation in moisture content of the twigs of trees during the winter. Water loss through transpiration, absorption of moisture from the atmosphere, water conduction through the wood, loss of dry weight by respiration during the winter, and translocation of material would influence the relative proportion of moisture in the various complex tissues as expressed in per cent dry weight.

As early as 1875, Wiesner and Pacher proved that 1-5 year horse-chestnut twigs transpired an appreciable amount of moisture in winter at a temperature of 13.17 degrees C., and a slight amount also at —30 degrees C. Table III is adapted from Wiesner and Pacher, showing the amount of water loss after 24-hour, weekly, and monthly periods at a temperature of 13.5 to 17.5 degrees C. The figures show conclusively that leaf traces favor transpiration as compared with the neighboring periderm; and that one-year portions of twigs are protected against transpiration through the periderm to a greater extent than 2- and 3-year twigs.

TABLE III

Loss of Water from Horse-Chestnut Twigs *(Aesculus hippocastanum)*, January, University of Vienna

Expressed in per cent of the Original Weight of the Twig

(Adapted from Wiesner and Pacher, 1875)

	1-Year twigs		2-Year twigs		3-Year twigs		5-Year twigs
	No leaf traces	Leaf traces present	No leaf traces	Leaf traces present	No leaf traces	Leaf traces present	Leaf traces not distinguishable
After	0.836	1.583	0.965	1,268	1.105	1.206	0.641
24-hour	.836	1.531	1.081	1.326	1.126	1.242	.641
periods	.836	1.688	1.390	1.563	1.294	1.228	.603
	.662	1.490	1.181	1.312	1.094	1.152	.549
	.767	1.320	1.043	1.238	1.043	1.052	.573
	.550	1.295	0.965	1.230	1.021	1.053	.505
	0.650	1.343	1.236	1.239	1.023	1.075	0.493
After one week	5.122	10.250	7.861	9.76	7.706	8.008	4.005
After 30 days	16.376	31.741	23.551	28.879	23.837	25.219	11.407

The work of R. Hartig (1883) showed that twigs of deciduous trees and coniferous evergreens lose perceptible quantities of water when exposed outdoors in April. He also observed that these twigs gained in moisture during rainy weather. These results were verified by Kny (1895), who found that defoliated twigs lost water by transpiration, but may also absorb water slowly from the atmosphere. Bailey (1896) reported that apple twigs lose appreciable amounts of moisture in winter in western New York. The work of Sandsten (1904), at Madison, Wisconsin, established the fact that the loss of moisture varied from about 2 per cent to nearly 15 per cent in the two observed winters when 8-year-old apple trees were cut off, exposed outdoors, and weighed at intervals during the winter, December to April.

The recent work of Hildreth (1926) at St. Paul, Minnesota, on winter dessication of severed terminal apple twigs, sealed with grafting wax on the cut surface and exposed during the winter and weighed outside at intervals, shows that the twigs of the two varieties used in the experiment lost weight during practically the entire period. The

only break occurred after a rain in February when a sample which had lost more moisture by reason of longer exposure, gained weight. This gain in weight during rainy weather is in harmony with the results of R. Hartig (1883) and Kny (1895). Hildreth reports that the rate of loss was gradual during periods of low temperature, but increased as spring opened despite the fact that the moisture content of the shoots was being constantly diminished. Except for the rainy period in February, the evaporation curve of twigs agrees fairly closely with the temperature curve. "Wealthy" twigs outdoors lost 29.3 per cent of the original weight between January 1 and April 8, altho nearly two-thirds of this loss occurred during the last month.

Sachs (1860a) pointed out that the freezing of the soil retards moisture absorption by trees in winter. According to Müller-Thurgau (1886), the soil and the woody stem of the tree in winter are poor conducting channels and unable to make good the water lost by evaporation from ice crystals in the tissues. This apparently explained to him the fact that twigs of apple and other trees lost weight when exposed outdoors on two consecutive cold nights. That roots exert only a minor influence on the water content of leaves in winter, is indicated by the experiments of Ivanoff (1924), at Petrograd (Leningrad), with evergreen trees cut from the roots. According to this worker the relatively low temperature of the surface soil and the atmosphere tend to slow down the conduction of water in the wood of trees in winter. Conduction does not cease entirely, but is slowed down so greatly that the water supplied is not sufficient to cover the loss by transpiration. This condition leads to a marked decrease in moisture content of the tree.

Kosaroff (1901), at Sofia, reported experiments that pointed to the fact that leafless twigs of trees both absorbed and transpired considerable quantities of water during winter at temperatures down to 0 degrees C. Therefore there must be movement of water in the twig. This conclusion seems in harmony with the work of Hildreth (1926) with apple twigs. "McIntosh" twigs severed from the tree, sealed on the cut surface, and exposed outdoors from December 17 to March 3, showed a moisture content of 34.5 per cent; the shoots left on the tree contained 45.5 per cent moisture on the same date. Hildreth concludes that trees in winter may be able to make up in part the moisture loss occurring from evaporation. He explains, however, that the temperature of the air and possibly of trunk and branches was frequently above freezing during the period.

Another factor influencing the relative proportion of moisture in the twigs of trees as expressed in per cent dry weight is the loss of

combustible material in respiration. A decrease of dry weight without a proportionate decrease in moisture apparently indicates an increase in moisture content. That terminal apple twigs lose a significant amount of their dry weight by respiration during the winter is shown by the work of Beaumont and Willaman (1924), at St. Paul, Minnesota. These workers measured the amount of CO_2 given off by terminal apple twigs on the tree in February and March. De Long (1927), working in the same locality with excised apple twigs, reports similar results. The table below shows the amount of CO_2 given off on the basis of 24-hour periods, per 100 grams of material:

Temperature degrees C.	CO_2 given off mg.
−6	12
+6	48

Still another factor that may have an appreciable effect upon the relative proportion of moisture in the twigs of trees as expressed on a dry weight basis should be mentioned. There is the possibility of a change in dry weight taking place as a result of the translocation of material to or from a region during the winter season. There is, however, apparently no information available on the subject of the transport of material in dormant twigs during the winter.

The work of the authorities quoted shows that at least five factors may be operative during the winter season that might influence a change in the moisture content of woody twigs as expressed on a dry weight basis: (1) loss of water by transpiration, (2) a tendency to make good this loss in part by conduction of moisture through the wood from the root, (3) a tendency to absorb moisture from the atmosphere, especially in rainy weather, (4) diminution of total dry weight by respiration, and (5) increase or decrease in dry weight due to translocation of material during the winter. The moisture relations of the complex tissues of the apple twig during the winter must be interpreted in the light of these dynamic changes.

During the dormant season, end of October to April, there is a gradual to rapid decline in moisture in the various tissues, depending upon the nature of the complex tissue, and later a rise in two of the complex tissues.

In taking up the consideration of complex tissues from the outside to the center of the twig, we notice that in the periderm there is a sharp falling off in moisture content throughout the whole dormant period. The rate of loss is slowed down somewhat after the latter part of December. This continued moisture loss is probably due to the fact that the phellem is gradually dying or is dead tissue during this period. There is a rapid rise in March and April with the renewed activity of the phellogen.

After active growth ceases in the fall, the cortex-phloem is on the decline during the winter. This may have a possible bearing upon the moisture relations of this complex tissue. The cortex-phloem shows first a marked decrease (40.15 per cent on dry weight basis) in moisture content to the end of January and then an equally rapid increase in moisture content through February and March.

When the woody tissues are considered, a fairly gradual decline in moisture content of the outer xylem is noticed until March. The inner xylem-pith shows a fairly important loss of moisture until the latter part of December and then a fairly important gain until the end of February. During the whole period the cortex-phloem has a higher and the periderm a lower moisture content than the xylem tissues. The fall in moisture content from October to December or January may be due to water loss by transpiration, as explained by Ivanoff (1924). With the lowering of the temperature to the minimum in January, water conduction is slowed down so greatly that the moisture supplied is not sufficient to cover the loss by transpiration, leading to a marked decrease in moisture content in the complex tissues. Altho the dry weight is cut down by respiration during the same period, the experiments of Beaumont and Willaman (1924) and De Long (1927) show that the amount of CO_2 respired decreases markedly with the lowering of the temperature. The net result is therefore a decrease in moisture on a dry weight basis in spite of an actual decrease in dry weight. The increase in moisture in the cortex-phloem through February is probably due to increased water conduction with the gradual increase in temperature. Water absorption from the atmosphere may also influence the moisture content of the twig, as emphasized by R. Hartig (1883) and Kny (1895), but it is impossible to estimate the relative importance of this factor without further experimentation. The relatively greater losses or gains in moisture by the periderm and cortex-phloem may be due to their greater exposure to external conditions, or to the nature of the tissues, as has been suggested.

These individualities of the separate complex tissues, however, naturally disappear when the moisture is expressed as per cent of dry weight of the entire twig. On this basis the results agree in general with those of Hildreth (1925) with terminal apple twigs of "Oldenburg," the winter-hardy parent of "University." Hildreth made his observations in the same locality in 1923-24. There is a very gradual decline of moisture until the end of January, and then a very gradual increase to the end of February. Between the end of October and the end of January there is a maximum fall of a little over 10 per cent on a dry weight basis. This harmonizes with the results of Sandsten (1904). From the end of January to the end of February there is a rise of a little less than 2 per cent. From the end of December to the

end of February the moisture is, therefore, practically constant when expressed on a dry weight basis.

It was emphasized earlier in this paper that the variation in the relative proportions of the tissues made it impossible to obtain a true conception of the moisture relations by analyzing the entire twig. There is considerable variation in the relative proportions of the complex tissues, as shown in Figure 4. On the basis of the entire twig, the picture presented follows that of the outer xylem fairly closely. The rapid fall and rise of moisture in the cortex-phloem during the winter and the important continual decline of moisture in the periderm, are entirely masked.

Transition Between Dormant and Growing Periods

During the transition between the dormant and the growing periods, in March and April, before active growth begins, there is a general tendency for the cortex-phloem and xylem tissues to gain moisture after the latter part of March and early April. The periderm shows a continuous gain from the beginning to the end of the period.

The marked rise in moisture content begins in early April before there is much rainfall, for only 0.39 inch of precipitation is recorded between April 4, the date of minimum moisture content for the cortex-phloem and xylem tissues, and April 18, when the marked rise in moisture is already fairly under way, as shown in Table II and Figure 5. At this time the reserve of moisture in the soil is probably sufficiently abundant to supply all the needs of the tree. The temporary decline at the end of March is, therefore, apparently not bound up with the absolute amount of rainfall, but is dependent on the chemical and physical transformations in the twig.

REGIONAL AND SEASONAL DISTRIBUTION OF CARBOHYDRATES

Microchemical determinations of the various carbohydrate fractions of the tissues of woody plants have been made quite extensively in the past. Macrochemical analyses of the chemical constituents of the complex tissues of such plants are, however, apparently rare and fragmentary. Niklewski (1906), at Leipzig, analyzed the cortex-phloem and wood of various woody plants for ether extract and sugars in an attempt to determine the rôle of fat in the transformations taking place during the winter period. Proebsting (1925) analyzed the cortex-phloem and xylem tissues of apple twigs for certain carbohydrate fractions and nitrogen from April to September, in making a study of the relation of stored food to cambial activity.

This section is devoted to a consideration of the evidence showing variation in the reserve carbohydrate constituents in the four complex

tissues of the 2-3 year portions of the apple twig—periderm, cortex-phloem, outer xylem, and inner xylem-pith. For comparative purposes these data have also been calculated on the basis of the entire twig.

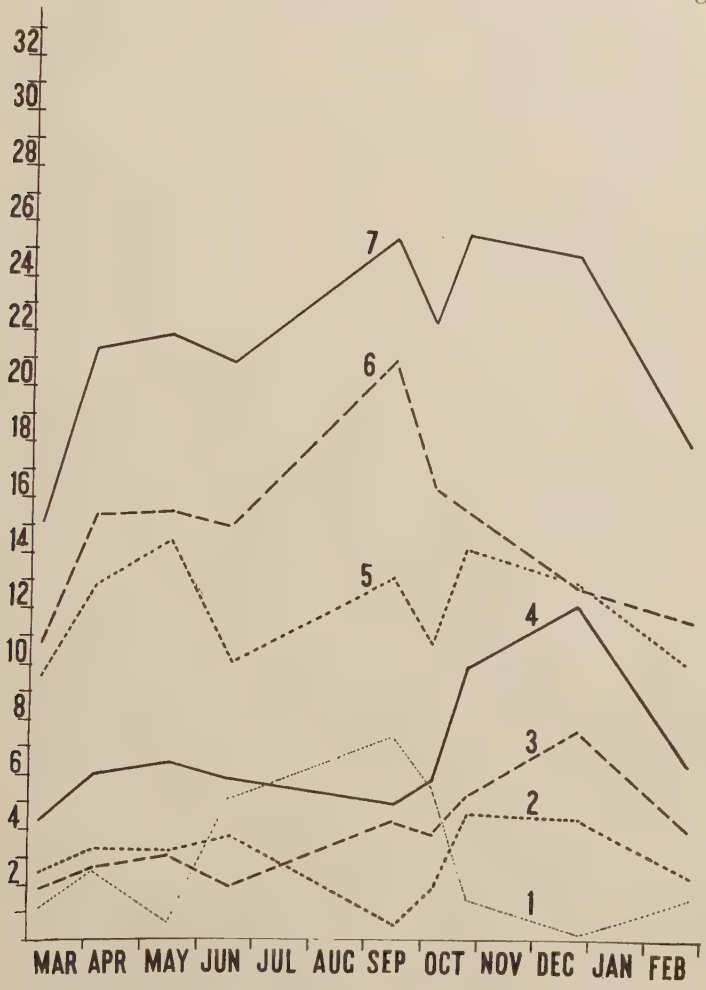

Fig. 6. Seasonal distribution of carbohydrates in periderm; acid-hydrolyzable hexosans (1); non-reducing sugars (2); reducing sugars (3); total sugars (4); pentosans (5); acid-hydrolyzable alcohol-insoluble carbohydrates (6); and total carbohydrates, except cellulose (7). Expressed in per cent dry weight.

The results of the macrochemical analyses—total carbohydrates, acid-hydrolyzable hexosans, reducing sugars, non-reducing sugars, and total sugars—are given in Tables IV, V, VI, VII, and VIII on a dry weight basis. In Table IX the total sugars, acid-hydrolyzable hexosans, and pentosans are expressed in per cent total carbohydrates except cellulose. The data are shown graphically in Figures 6 to 13.

TABLE IV

REGIONAL AND SEASONAL DISTRIBUTION OF SUGARS IN 2-3 YEAR PORTIONS OF APPLE TWIGS, MARCH 7, 1925, TO FEBRUARY 26, 1926

Expressed in per Cent Dry Weight

Date	Periderm	Cortex-phloem	Outer xylem	Inner xylem-pith	Whole twig
		Total Sugars			
March 7	4.39	9.82	3.33	3.23	4.50
April 4	6.00	5.51	1.20	1.37	2.45
May 16	6.39	4.12	0.89	1.06	2.02
June 20	5.93	5.61	0.81	0.83	1.97
September 17	4.90	5.46	0.57	0.71	1.67
October 10	5.87	7.42	0.91	0.96	2.51
October 23	9.83	12.07	1.74	2.27	3.89
December 23	12.04	13.17	5.86	8.11	8.31
February 26	6.37	8.87	4.29	6.16	6.49
		Reducing Sugars			
March 7	1.94	6.32	1.12	1.62	2.23
April 4	2.62	2.86	0.71	0.62	1.24
May 16	3.14	2.49	0.50	0.77	1.18
June 20	2.08	3.82	0.40	0.70	1.15
September 17	4.24	4.02	0.34	0.44	1.19
October 10	3.92	4.80	0.46	0.62	1.58
October 23	5.18	8.01	0.70	0.81	2.09
December 23	7.60	8.93	2.49	3.68	4.35
February 26	3.99	5.54	1.80	2.78	3.24
		Non-Reducing Sugars			
March 7	2.45	3.50	2.21	1.61	2.27
April 4	3.38	2.65	0.49	0.75	1.21
May 16	3.25	1.63	0.39	0.29	0.84
June 20	3.85	1.79	0.21	0.13	0.82
September 17	0.66	1.44	0.23	0.27	0.48
October 10	1.95	2.62	0.45	0.34	0.93
October 23	4.65	4.06	1.04	1.46	1.80
December 23	4.44	4.24	3.37	4.43	3.96
February 26	2.38	3.33	2.49	3.71	3.25

TABLE V

REGIONAL AND SEASONAL DISTRIBUTION OF ACID-HYDROLYZABLE HEXOSANS IN 2-3 YEAR PORTIONS OF APPLE TWIGS, MARCH 7, 1925, TO FEBRUARY 26, 1927

Expressed in per Cent Dry Weight

Date	Periderm	Cortex-phloem	Outer xylem	Inner xylem-pith	Whole twig
March 7	1.19	3.22	7.27	9.00	6.60
April 4	2.59	4.74	7.08	10.29	7.22
May 16	1.04	2.71	0.99	6.76	2.94
June 20	4.98	4.46	5.69	8.29	6.26
September 17	7.36	6.18	4.84	5.23	5.31
October 10	5.61	7.08	5.77	3.95	5.36
October 23	1.49	1.30	7.01	7.77	6.14
December 23	0.24	0.69	3.13	3.16	2.53
February 26	1.53	2.36	4.52	5.06	4.54

TABLE VI

REGIONAL AND SEASONAL DISTRIBUTION OF PENTOSANS IN 2-3 YEAR PORTIONS OF APPLE TWIGS, MARCH 7, 1925, TO FEBRUARY 26, 1926

Expressed in per Cent Dry Weight

Date	Periderm	Cortex-phloem	Outer xylem	Inner xylem-pith	Whole twig
March 7	9.53	13.66	20.49	16.81	17.29
April 4	12.84	11.04	22.07	20.70	18.82
May 16	14.49	14.74	23.56	21.17	20.40
June 20	10.02	14.02	22.02	22.52	19.94
September 17	13.14	14.02	22.91	22.93	20.91
October 10	10.74	12.17	22.35	23.25	19.87
October 23	14.19	16.53	21.30	20.80	20.11
December 23	12.47	15.82	22.10	21.84	20.30
February 26	9.99	15.12	19.84	21.78	20.90

TABLE VII

REGIONAL AND SEASONAL DISTRIBUTION OF ACID-HYDROLYZABLE ALCOHOL-INSOLUBLE CARBOHYDRATES (ACID-HYDROLYZABLE POLYSACCHARIDES) IN 2-3 YEAR PORTIONS OF APPLE TWIGS, MARCH 7, 1925, TO FEBRUARY 26, 1926

Expressed in per Cent Dry Weight

Date	Periderm	Cortex-phloem	Outer xylem	Inner xylem-pith	Whole twig
March 7	10.72	16.88	27.76	25.81	23.89
April 4	15.41	15.78	29.15	30.99	26.04
May 16	15.53	17.45	24.55	27.93	23.34
June 20	15.00	18.48	27.71	30.81	26.20
September 17	20.50	20.20	27.75	28.16	26.22
October 10	16.35	19.25	28.12	27.20	25.23
October 23	15.68	17.83	28.31	28.57	26.25
December 23	12.71	16.51	25.23	25.00	22.83
February 26	11.52	17.48	24.36	26.84	22.87

TABLE VIII

REGIONAL AND SEASONAL DISTRIBUTION OF ACID-HYDROLYZABLE ALCOHOL-INSOLUBLE CARBOHYDRATES PLUS TOTAL ALCOHOL-SOLUBLE CARBOHYDRATES IN 2-3 YEAR PORTIONS OF APPLE TWIGS, MARCH 7, 1925, TO FEBRUARY 26, 1926

Expressed in per Cent Dry Weight

Date	Periderm	Cortex-phloem	Outer xylem	Inner xylem-pith	Whole twig
March 7	15.11	26.70	31.09	29.04	28.39
April 4	21.41	21.29	30.35	32.36	28.49
May 16	21.92	21.57	25.44	28.99	25.36
June 20	20.93	24.09	28.50	31.64	28.17
September 17	25.40	25.66	28.32	28.87	27.89
October 10	22.22	26.67	29.03	28.16	27.74
October 23	25.51	29.90	30.05	30.84	31.14
December 23	24.75	29.68	31.09	33.11	31.14
February 26	17.89	26.35	28.65	33.09	29.36

TABLE IX

REGIONAL AND SEASONAL DISTRIBUTION OF TOTAL SUGARS, PENTOSANS, AND ACID-HYDROLYZABLE HEXOSANS, IN 2-3 YEAR PORTIONS OF APPLE TWIGS, MARCH 7, 1925, TO FEBRUARY 26, 1926

Expressed in per Cent of Total Reserve Carbohydrates

Date	Periderm	Cortex-phloem	Outer xylem	Inner xylem-pith	Whole twig
		Total Sugars			
March 7	29.05	36.77	10.71	11.12	15.85
April 4	28.02	25.88	3.95	4.23	8.59
May 16	29.15	19.10	3.49	3.65	7.96
June 20	28.33	23.28	2.84	2.62	6.99
September 17	19.29	21.27	2.01	2.45	5.98
October 10	26.41	27.82	3.13	3.40	9.04
October 23	38.53	40.37	5.79	7.36	12.49
December 23	48.64	44.37	18.84	26.06	26.68
February 26	35.60	33.66	14.97	18.61	22.10
		Pentosans			
March 7	63.08	51.16	65.90	57.88	60.83
April 4	59.97	51.85	72.71	63.96	66.05
May 16	66.10	68.33	92.61	73.02	80.44
June 20	47.87	58.19	77.26	71.17	70.78
September 17	51.73	54.63	80.89	79.42	74.97
October 10	48.33	45.63	76.98	82.56	71.62
October 23	55.62	55.28	70.88	67.44	64.57
December 23	50.38	53.30	71.08	70.08	65.18
February 26	55.84	57.38	69.24	65.82	71.18
		Acid-Hydrolyzable Hexosans			
March 7	7.87	12.05	23.38	30.99	23.24
April 4	12.09	22.26	23.32	31.79	25.34
May 16	4.74	12.56	3.89	23.31	11.59
June 20	23.79	18.51	19.96	26.20	22.22
September 17	28.97	24.08	17.09	18.11	19.03
October 10	25.24	26.54	19.87	14.02	19.32
October 23	5.84	4.34	23.32	25.19	19.71
December 23	0.96	2.32	10.06	10.15	8.15
February 26	8.55	8.95	15.77	15.29	15.46

Total Carbohydrates

The total amount of reserve carbohydrates in 2-3 year portions of apple twigs is quite large, as shown in Table VIII and Figures 6 to 10 inclusive. The magnitude of this reserve material is apparently directly proportional to the nearness to the center of the twig. The highest per cent, from 28.16 to 33.11 on a dry weight basis, is found in the inner xylem-pith. The percent in the outer xylem, from 25.44 to 31.09, is only slightly lower than that of the tissue which it surrounds; the per cent in the cortex-phloem, from 21.29 to 29.90, is lower than that of the outer xylem, and the corresponding value for the periderm is below that of the cortex-phloem.

On the basis of the entire twig, the tendency of the outer xylem is reflected except from September to December, when the high carbohydrate content of the cortex-phloem makes possible a maximum in October for the twig as a whole. This is shown in Table VIII and Figure 10.

At the end of the dormant season there is a marked decline in the total reserve carbohydrates in most of the complex tissues of 2-3 year portions of apple twigs. In the cortex-phloem there is a rapid decline after the latter part of December, in the outer xylem the decline

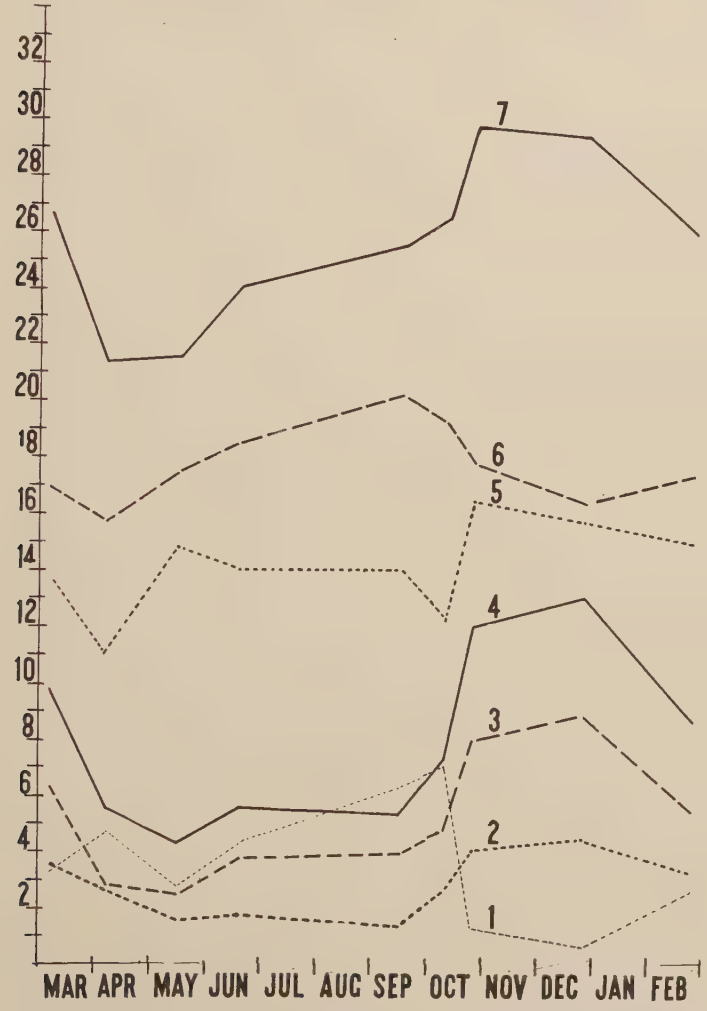

Fig. 7. Seasonal distribution of carbohydrates in cortex-phloem; acid-hydrolyzable hexosans (1); non-reducing sugars (2); reducing sugars (3); total sugars (4); pentosans (5); acid-hydrolyzable alcohol-insoluble carbohydrates (6); and total carbohydrates, except cellulose (7). Expressed in per cent dry weight.

is steady but less marked, and in the inner xylem-pith there is a rise during March that is probably due to translocation of reserve carbohydrates from other parts of the tree and a lesser or later utilization of stored materials. The minimum in reserve carbohydrates is

reached during April and May for the cortex-phloem and about the middle of May for the outer xylem and inner xylem-pith.

The periderm shows a difference in trend of reserve carbohydrates at the end of the dormant season and at the time of the initiation of growth in spring. There is a rise until the middle of May with a slight

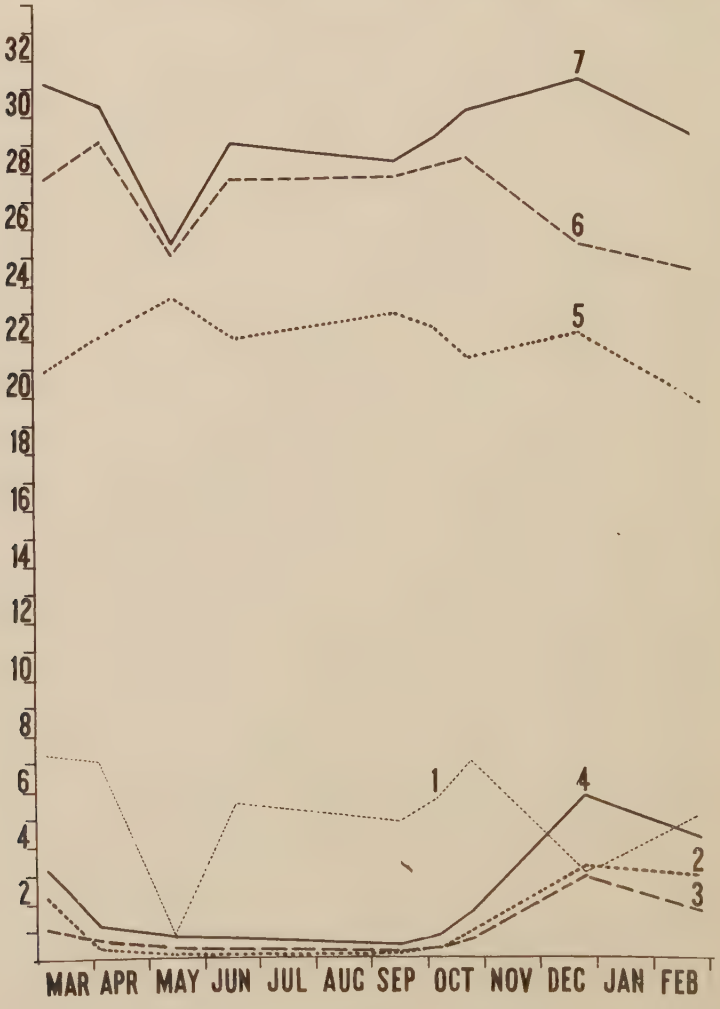

Fig. 8. Seasonal distribution of carbohydrates in outer xylem; acid-hydrolyzable hexosans (1); non-reducing sugars (2); reducing sugars (3); total sugars (4) pentosans (5); acid-hydrolyzable alcohol-insoluble carbohydrates (6); and total carbohydrates, except cellulose (7). Expressed in per cent dry weight.

decline in June. This is probably due to the nature of the periderm tissue, as explained in the section devoted to moisture distribution.

The marked decline in stored or reserve carbohydrates in all the complex tissues except the periderm, at the time growth is initiated,

is undoubtedly due to the utilization of the reserves in growth extension. This statement is in harmony with the work of Puriewitsch (1898), who maintains that organic materials move from the storage tissues to other regions when the transported materials are utilized in the tissues receiving them. Translocation of stored organic materials

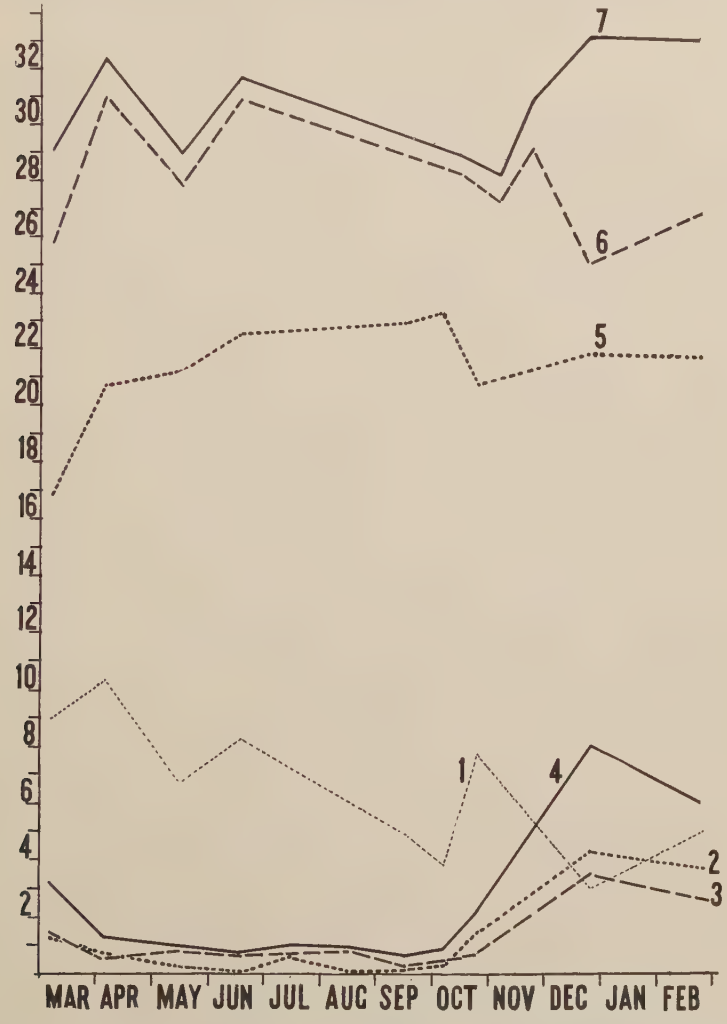

Fig. 9. Seasonal distribution of carbohydrates in inner xylem-pith; acid-hydrolyzable hexosans (1); non-reducing sugars (2); reducing sugars (3); total sugars (4); pentosans (5); acid-hydrolyzable alcohol-insoluble carbohydrates (6); and total carbohydrates, except cellulose (7). Expressed in per cent dry weight.

from a region also takes place when like organic substances move through the region to more distant consuming tissues.

The following phenological data are of interest in this connection:

Date	Development of "University" Apple Trees, Spring 1925
March 23	Trees dormant
April 4	Fruit buds beginning to swell
April 18	Fruit buds partially expanded
May 2	Fruit buds fully expanded; flower petals pink, and leaves ¾ inch wide
May 16	Trees in full bloom; new terminal branches 3 inches long.

Reference to Table VIII and Figures 6, 7, 8. and 9 reveals the fact that as the development of new shoots progresses the total reserve carbohydrates in the complex tissues decrease toward a minimum except in the periderm, which seems to draw on the carbohydrate supply at this time. When the fruit buds are beginning to swell, in early April, the reserve carbohydrates in the cortex-phloem have fallen to the minimum, as shown in Figure 7. When the trees are in full bloom and the new terminal shoots are 3 inches long, the reserve carbohydrates in the xylem tissues have declined to a minimum by the middle of May, as indicated in Figures 8 and 9.

The marked increase in total reserve carbohydrates in all the complex tissues except the periderm, after the middle of May, is ascribable to the net gain from the photosynthetic process in the leaves. Surplus carbohydrates are transported to the stem and accumulate as reserves.

In the periderm there is a rapid building up of reserve carbohydrates after the latter part of June. In the cortex-phloem the rise of carbohydrates is consistent during the entire growing season; in the outer xylem there is a very slight decline to September after a maximum is reached in June. The inner xylem-pith follows the trend of the outer xylem, but the decline from June to September is more marked.

The transition between the growing season and the dormant period, September to October, is characterized by carbohydrate accumulation in the various complex tissues. From the standpoint of total carbohydrates, Combes and Kohler (1922) have shown that from 1/5 to 1/4 of the soluble carbohydrates in the leaves is transported to the living parts to build up a reserve. The translocation of this material is reflected in a very marked increase in the cortex-phloem and the inner xylem-pith, a rise of 4.24 per cent from September 17 to October 23 in the cortex-phloem, and of 4.95 per cent from October 10 to September 23, in the inner xylem-pith. In the cortex-phloem the maximum is reached in the latter part of October, but in the xylem tissues the maximum is not reached until December. Apparently considerable time elapses after the leaves have fallen before translocation to the various storage tissues is completed.

After the maximum is reached, in October or December, there is a decline in carbohydrates, the rate varying with the particular complex tissue.

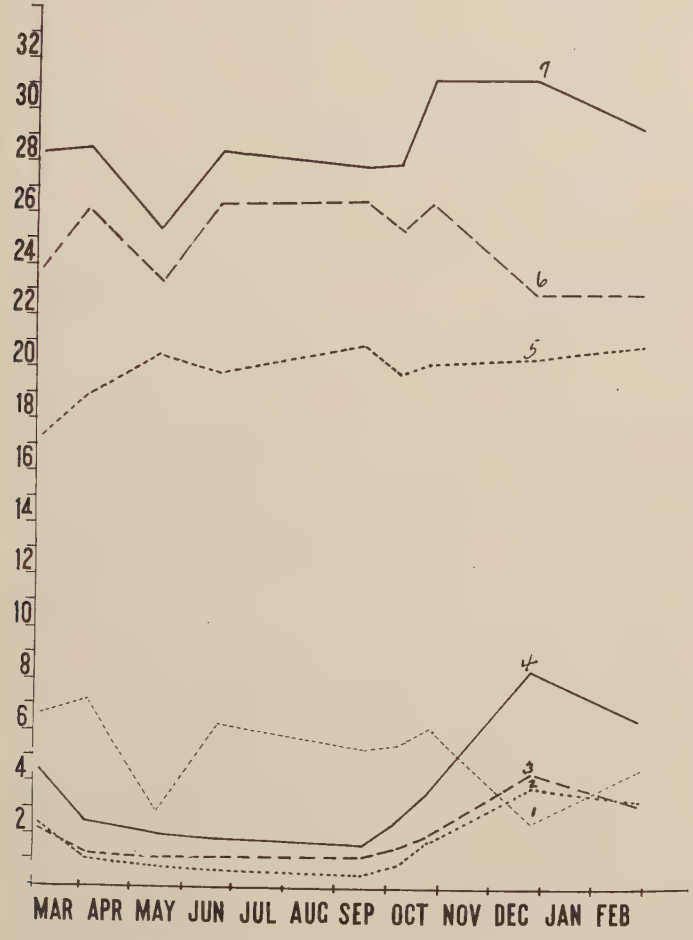

Fig. 10. Seasonal distribution of carbohydrates in the entire 2-3 year portion of the twig; acid-hydrolyzable hexosans (1); non-reducing sugars (2); reducing sugars (3); total sugars (4); pentosans (5); acid-hydrolyzable alcohol-insoluble carbohydrates (6); and total carbohydrates, except cellulose (7). Expressed in per cent dry weight.

In the periderm there is first a gradual decline during November and December, but from January on there is a rapid decline to a minimum in March. In the cortex-phloem the decline is very gradual between October and January, but the rate increases from this time until the minimum is reached, in May. In the outer xylem there is a marked decline from the December maximum to the May minimum.

In the inner xylem-pith there is first a very gradual decline from the December maximum to the end of February, and then a rise to a new maximum in March-April preceding the period of rapid growth extension, during which the minimum is reached in May.

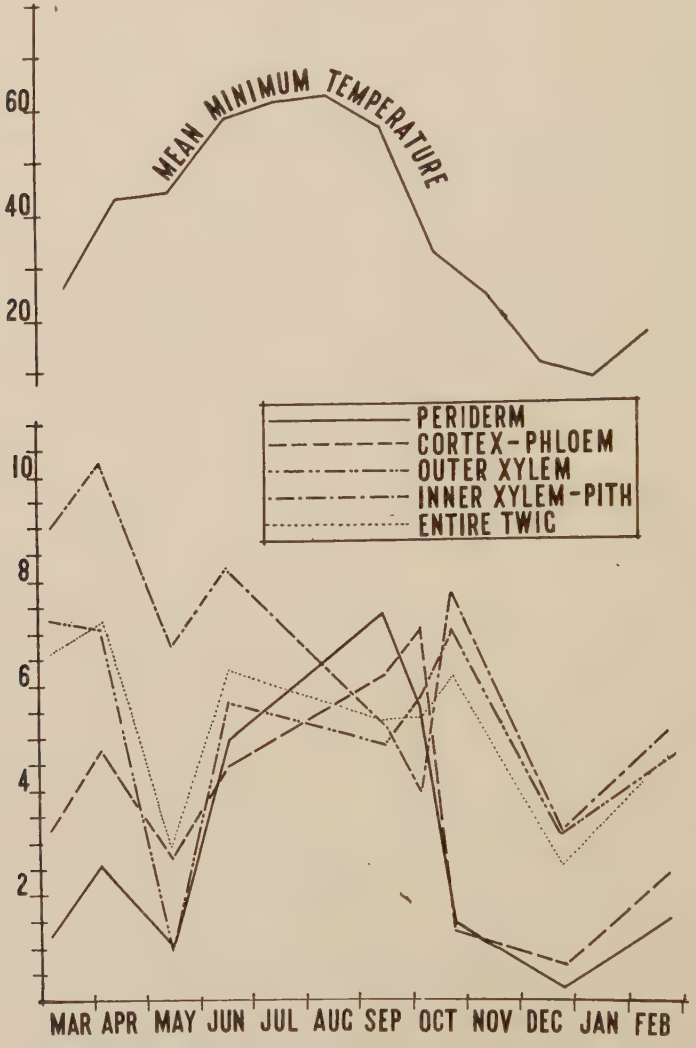

Fig. 11. Lower, seasonal distribution of acid-hydrolyzable hexosans in periderm, cortex-phloem, outer xylem, and inner xylem-pith. Expressed in per cent dry weight.
Upper, mean monthly minimum temperature, expressed in degrees Fahrenheit.

The decline in carbohydrates during the winter is apparently due largely to loss by respiration, as shown by the work of Beaumont and Willaman (1924) and De Long (1927). As the temperature begins to rise in February and March from the low level of December and

January, part of the rapid decline is probably due to loss through translocation of materials to the growing points.

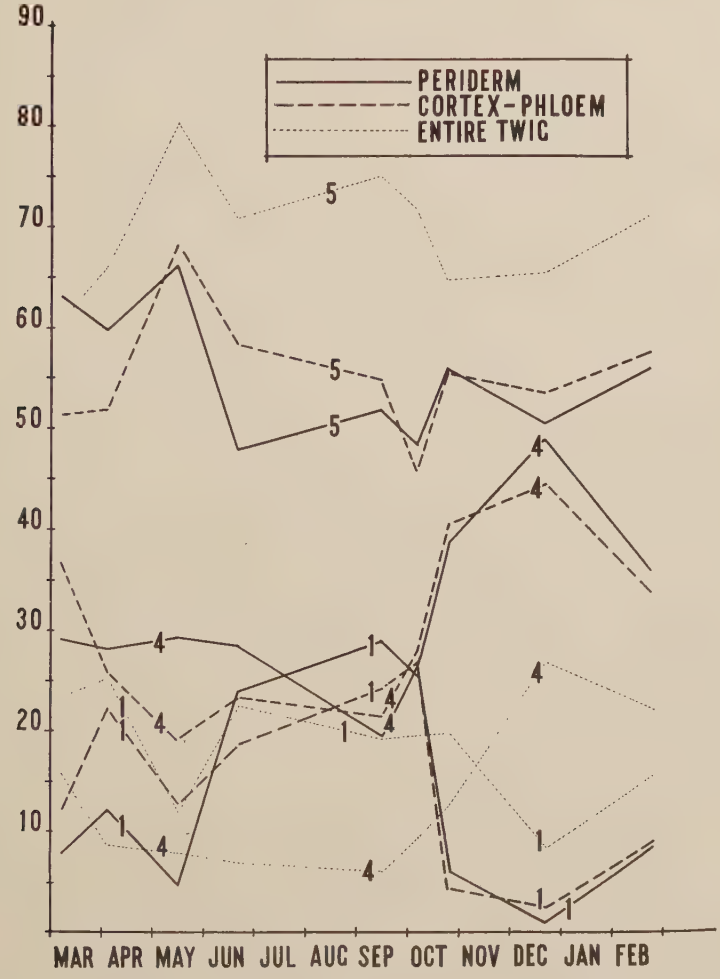

Fig. 12. Seasonal distribution of acid-hydrolyzable hexosans (1); total sugars (4); and pentosans (5), in periderm, cortex-phloem, and entire twig. Expressed in per cent of total carbohydrates.

Pentosans

A large proportion of the reserve carbohydrates of the complex tissues of 2-3 year portions of apple twigs consists of pentosans, as shown in Table IX and Figures 6 to 10, inclusive. The pentosan content of the xylem tissues, always over 55 per cent, is greater than that of the periderm and cortex-phloem, always over 45 per cent of the total reserve carbohydrates.

On the basis of total dry weight, there appear to be marked fluctuations in the pentosan content of the complex tissues of 2-3 year portions of apple twigs. At certain times of the year these fluctuations are, however, apparent rather than real. The rise in pentosans from April to May in all the tissues represents a relative diminution

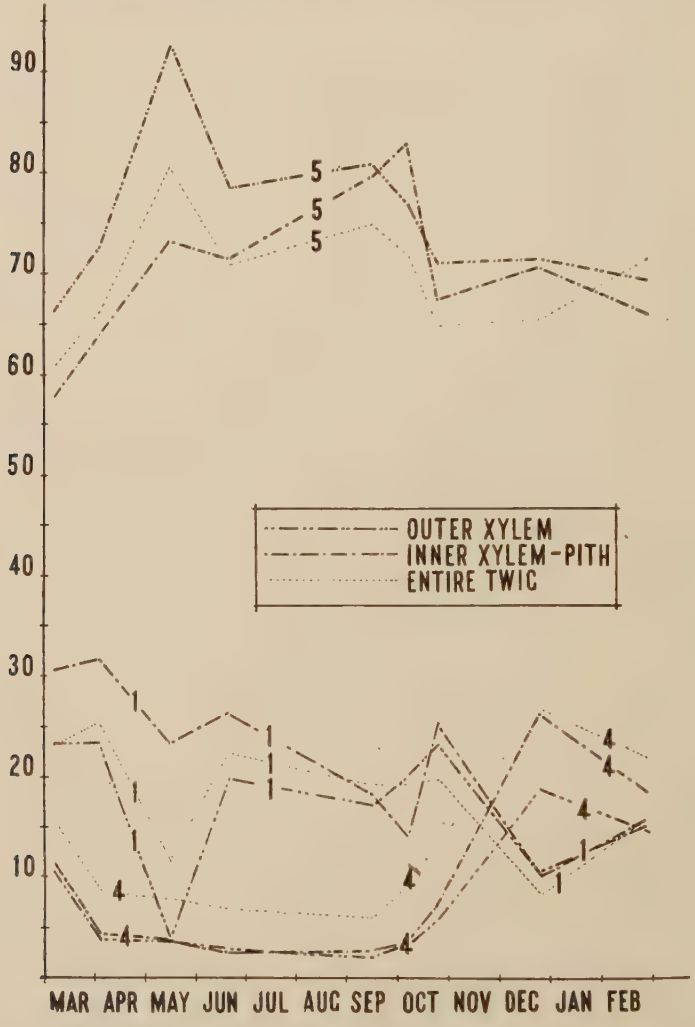

Fig. 13. Seasonal distribution of acid-hydrolyzable hexosans (1); total sugars (4); and pentosans (5), in outer xylem, inner xylem-pith, and entire twig. Expressed in per cent of total carbohydrates.

in the hexosans, as shown in Table IX and Figures 12 and 13, where the pentosans are expressed in per cent of total reserve carbohydrates.

On the basis of total reserve carbohydrates, the relative proportion of pentosans in periderm and cortex-phloem remains fairly constant

after the apparent spring rise in April-May as shown in Figure 12, except for the decline in September-October. In the xylem tissues the decline in pentosan content on the basis of total reserve carbohydrates does not take place until October. Between October and March this lower level is maintained.

The pentosan content is probably fairly constant. The apparent decline of pentosans in the woody tissues from October to March is due to the relatively great increase in total sugars preceding leaf-fall.

Acid-Hydrolyzable Hexosans and Sugars

The acid-hydrolyzable hexosans and sugars presumably play an important rôle in the economy of the apple tree. These are apparently the most labile types of reserve carbohydrates in the plant cell, and are probably in equilibrium with other forms of carbohydrates, especially sugars, changing from one to the other in response to such varying external and internal environmental conditions as changing concentrations of sugar solution, varying temperatures, presence of enzymes.

Sugars: Growing Period, April to September

During the growing season the sugar content is extremely small in the xylem tissues, always below 2 per cent between April 4 and October 10, but the amount of sugars is relatively much more abundant in the periderm and cortex-phloem, ranging from 4.90 to 6.00 and 4.12 to 7.42 per cent on a dry weight basis, during the same period, as shown in Table IV and Figures 6, 7, 8, and 9. On the basis of per cent total carbohydrates, the same relationship exists. There is a slight drop in the sugar content of the periderm from May to September. In the cortex-phloem, after the spring drop in April and May there is a rise to a new level in June. This level remains almost constant until September, when a rise to a new higher level begins.

The sugar content of the xylem tissues is relatively constant during the entire growing season, usually below 1 per cent until September, when a marked increase sets in.

In the periderm the reducing sugars are greater in amount than the non-reducing sugars after the middle of July; and in the cortex-phloem, outer xylem, and inner xylem-pith the non-reducing sugars are consistently lower than the reducing sugars during the growing period, as shown in Table IV and Figures 6, 7, 8, and 9.

Acid-Hydrolyzable Hexosans: Growing Period, April to September

In March-April, just before active growth begins, an acid-hydrolyzable hexosan maximum is attained, but with the beginning of active growth a marked decline begins, owing to the utilization of the hydrolyzed hexosans in growth extension. When the minimum hexosan content was reached, in the middle of May, the trees were in full bloom,

and the new shoots 3 inches long. From this time forward the photosynthetic function of the leaves of the new shoots apparently not only produces sufficient carbohydrates for further growth extension but also furnishes a net gain to be stored in the form of hexosans, which rise markedly in all the tissues, as shown in Figures 6, 7, 8, 9, and 11. That this represents a relative gain as compared with other reserve carbohydrate fractions is shown in Figures 12 and 13, where hexosans are expressed in per cent total carbohydrates.

After the middle of June there is a steady increase in hexosans to a fall maximum in periderm and cortex-phloem in September-October. There is, however, a falling off of hexosan content in the woody tissues during the same period. That this is an actual falling off in relation to the other reserve carbohydrates is shown in Figure 13. The decline is relatively greater in the inner xylem-pith.

Acid-Hydrolyzable Hexosan-Sugar Transformation: Dormant Period, September to March

The marked rise in total carbohydrates as a result of translocation from the leaves, as shown by Combes and Kohler (1922), is due in large measure to the relatively important increase in sugars, which reach a new maximum in December in all the complex tissues. During the same period there is an equally marked decline in acid-hydrolyzable hexosans, which reach a minimum in December for all the tissues. This shows that the rise of sugar is due to two causes: (1) the translocation of carbohydrates from the leaves and (2) the transformation of acid-hydrolyzable hexosans into sugar. Various workers have reported experiments that have a bearing on the cause and the extent of hexosan-sugar transformations.

Causal Mechanism

Einhoff (1805) demonstrated that in the potato tuber the sugar content increases with low temperature. Vogel (1820) reported experiments showing that after freezing and thawing starch paste loses its sticking quality and its fluidity. These properties are again taken on when the material is brought to the boiling point of water. He also showed that frozen starch paste loses considerable water by syneresis. These experiments were repeated by Sachs (1860b) in an attempt to come closer to an understanding of the effect of temperature upon organic substances in the cell.

Butkewitsch (1908) demonstrated a maltose-splitting enzyme in the cortex-phloem of *Sophora japonica, Rabinia pseudoacacia, Caragana arborescens,* and *Populus nigra.* He also prepared a similar enzyme from the wood of *Sophora japonica.* From this evidence Butkewitsch naturally concluded that the end product of starch splitting in trees is glucose.

Butkewitsch (1908) also showed that toluol and chloroform lower or inhibit the synthetic activity of the starch-building plastids. He found that lowering the temperature has a similar effect. He recognized the analogy between the processes, and ascribed the building up of sugar to the decrease in the ability of the plastids to form starch at lower temperatures.

Czapek (1901) proved that low temperature decreases the ability of the plastids to build starch. Higher sugar concentrations are required for the plastids to build starch at 0 degrees C. than at 16 to 18 degrees C. From this information the conclusion can be drawn that starch already stored may be reconverted into sugar as the result of an equilibrium reaction when the temperature is lowered.

Coville (1920) explained the starch-sugar transformation in plants on exposure to low temperature as an increased permeability of the starch grain to enzymes, thus allowing the hydrolytic catalyst to come in contact with the starch. He does not explain the regeneration of starch with the increase in temperature.

The effect of temperature on the acid-hydrolyzable hexosan content of the complex tissues of 2-3 year portions of apple twigs is shown in Table V and Figure 11. With the drop in mean minimum temperature, beginning in September, there is a profound change in the total amount of acid-hydrolyzable hexosans in all the complex tissues. The periderm and cortex-phloem show a rapid decline from October 10 to October 23, reaching a minimum in December. In the woody tissues there is a rise from October 10 to October 23. This is probably due to the fact that much carbohydrate material is being transported into the wood from the leaves, and the concentration of sugar may be high enough to cause a temporary deposition of starch. With the further lowering of the temperature this starch will be hydrolyzed to sugars because at lower temperatures higher concentrations of sugar are required for plastids to build starch, as demonstrated by Czapek (1901). Consequently, the starch minimum is reached in December-January with the mean minimum temperature at the lowest point. The increase in mean minimum temperature after the middle of January is accompanied by a corresponding rise in the acid-hydrolyzable hexosans, as shown in Figure 11.

Extent of Acid-Hydrolyzable Hexosan Disappearance

According to Theodor Hartig (1858), the building up of a starch reserve in the tree at the time of leaf-fall is to be considered as ended when the living cells of the wood and ray parenchyma of the cortex-phloem and pith are packed with starch grains. This starch maximum he considered as remaining unchanged until spring, followed at the beginning of summer by a starch minimum. This viewpoint was held

by prominent plant physiologists, including Pfeffer, until the 80's of the last century, in spite of the evidence to the contrary which accumulated after the 60's.

Famintzin and Borodin (1867) found low starch content in twigs of birch during winter. Twigs that were brought into the room built up starch in the same manner as twigs outside in the spring. These workers noticed that the starch disappears as growth begins, and expressed the opinion that starch formation is not due to translocation but is the result of the transformation of substances already present in the cells. Famintzin and Borodin frankly admitted that their experiments gave them no information as to what these substances are.

Reichardt (1871) noticed the absence of starch in winter (February) in single twigs of *Salix fusca, Tilia europaea,* and *Betula alba,* but he believed that these were exceptional cases, for he was dominated by the then prevalent theory that the reserve starch undergoes no transformation during the winter. He was apparently unaware of the work of Famintzin and Borodin (1867).

Russow (1882) at Dorpat, observed that little or no starch could be found in the cortex-phloem of many native trees and shrubs during the winter months (December, January, February), but oil or fat in the parenchymatous elements, which were packed with starch at the beginning and end of the growing period. In contrast, the parenchymatous elements of the wood were found to be as rich in starch in winter as at the end of the growing period.

Barenetzky and Grebnitzky (1884) explained that in certain trees there is a strach minimum in the cortex-phloem and wood during the winter, with a starch maximum in the fall and spring. According to these authorities the starch changes to oil in winter, and soft-wooded trees *(Tilia)* are entirely starch-free in winter; but in hard-wooded trees the starch disappears entirely only in the cortex-phloem. There is an abundance in the wood but the amount is somewhat reduced.

The classical work of Müller-Thurgau on the starch-sugar transformation in the potato with the lowering of the temperature appeared in 1882. Müller-Thurgau (1885) extended the theory built upon his work with the potato to explain the initiation and breaking of the rest period in trees. He maintained that "when the sugar transformation from the leaves stops then the development of the buds slows down and when the last sugar is transformed into starch then growth will cease. The rest period has begun. When in time starch is again changed to sugar by the action of low temperature then growth can take place again. Rest period is due to the fact that little or no sugar is available to the buds."

Alfred Fischer (1888, 1891), building upon the results of his own experiments, and those of his predecessors quoted above, but apparently unaware of the work of Famintzin and Borodin (1867), reported an extensive survey of the starch-oil and starch-glucose transformations in deciduous dicotyledonous and coniferous trees. On the basis of the principal reserve storage material during the winter, Fischer grouped trees into two classes. One group included trees in which the reserve starch that had accumulated during the growing season is changed to oil and to a lesser extent into glucose in the xylem and cortex-phloem at the beginning of winter. This group he named oil-storing trees or "Fettbäume" *(Betula, Tilia, Pinus sylvestris,* etc.). Another group included trees in which the reserve starch in the cortex-phloem is changed to glucose and perhaps another unknown substance, but the starch in the xylem-pith remains practically unchanged from fall to winter with slight fluctuations. This group he called starch-storing trees, or "Stärkebäume" *(Quercus, Ulmus, Acer, Pyrus, Sorbus,* etc.) In the "Stärkebäume," according to Fischer, there is but a very slight falling off of starch in the xylem-pith as compared with the fall maximum, so small, in fact, that all the rays may be colored black-blue with iodine during the winter months, and the starch disappears completely only in the cortex-phloem. In the cortex-phloem, Fischer recognized two starch maxima, fall and spring, and two starch minima, winter and May. In the xylem-pith he recognized only one starch minimum—in May—and a starch maximum from fall through the winter.

Niklewski (1906), at Leipzig, confirmed by means of macrochemical analyses the observations of Russow (1882) and Alfred Fischer (1891) on the starch-oil transformation in the "Fettbäume" during the winter.

The results, obtained by macrochemical analyses of 2-3 year portions of apple twigs, indicated in Tables IV, V, and IX, and Figures 6, 7, 8, 9, 11, 12, and 13, show that under central Minnesota conditions the acid-hydrolyzable hexosan content not only of the periderm and cortex-phloem but also of the woody tissues, is reduced materially during the winter, and that there is a marked rise in sugar during the same period. The results obtained in the case of one representative of the group of "Stärkebäume" do not show a high glucose content of the wood during the spring and summer nor a glucose minimum in the wood in winter, as reported by Fischer (1891). The difference in the results may be explained by a consideration of the technic used by Alfred Fischer in determining the glucose in the vessels of the wood. Fischer used his samples in the fresh condition or kept them in the dry state and soaked them with water before using. Twigs were split and put into a concentrated solution of copper sulphate for about 5 minutes; they were then rinsed with water and put into a boiling solution of Rochelle salt and sodium hydroxide for 2-5 min-

utes. The glucose reaction took place only in the outer layer of the wood, which was removed and the process repeated. Such a method evidently does not reach the glucose in the living cells and leaves non-reducing substances entirely out of account.

By the application of this method, Fischer found glucose relatively abundant in the vessels, or tracheids, of various kinds of trees during spring and summer, but relatively little or no glucose during winter. From the end of May to the starch minimum in the middle of the following May, he found abundant starch grains in the living cells of the wood, and therefore concluded that the starch of the xylem-pith does not change to sugar during the winter. Table V, however, shows that the hexosan content of the outer xylem is reduced almost by half between June 20 and December 23, and in the inner xylem-pith the reduction equals more than 50 per cent. Between October 23 and December 23 the decrease is even more marked. Figures 8 and 9 show that the sugars are correspondingly increased during the same period. These graphs also show that the non-reducing sugars are in excess over the reducing sugars in the xylem tissues. The fact that about 50 per cent of the hexosans remain unchanged in the xylem-pith during winter would tend to mask the situation so that microchemical observations apparently indicate that no change in starch content had taken place. Similarly, the glucose content of the tracheal vessels is probably so low in winter that the impression would be given that xylem tissues are poor in glucose. Fischer took no account of non-reducing sugars, which are in excess of glucose in the wood during winter in the particular apple trees under central Minnesota conditions. This explains the conclusion he arrived at and that he made the basis of his classification. If the facts shown in one of the "Stärkebäume" are representative of the rest of the group, it appears that the nomenclature adopted by Fischer is no longer adequate for the group as a whole. Sugar storage during the winter months is apparently more characteristic than hexosan storage in the xylem-pith as well as in the cortex-phloem of the particular apple trees under central Minnesota conditions. Figures 8, 9, 12, and 13 show conclusively that hexosan storage plays a minor rôle during winter as compared with the relative amount of total sugars present, in *Pyrus malus*. The term "Zuckerbäume," sugar-storing trees, appears to be a more appropriate name for these trees.

The facts presented show that the periderm and cortex-phloem differ from the outer xylem and inner xylem-pith with regard to acid-hydrolyzable hexosan maxima and minima. Table V and Figure 11 indicate two hexosan maxima and two minima for the periderm and cortex-phloem and three hexosan maxima and three minima for the outer xylem and inner xylem-pith.

Periderm and Cortex-Phloem

Fall maximum, September to October
Winter minimum, December to January
Spring maximum, March to April
Spring minimum, May

Outer Xylem and Inner Xylem-Pith

Summer maximum, June
Fall minimum, September to October
Fall maximum, October to November
Winter minimum, December to January
Spring maximum, March to April
Spring minimum, May

On the basis of the whole twig, as shown in Figure 10, the tendency of the outer xylem is reflected in the acid-hydrolyzable hexosan content, but the sugar content is greater than that of the outer xylem, owing to the high sugar content of the cortex-phloem.

REGIONAL AND SEASONAL DISTRIBUTION OF NITROGEN

Macrochemical studies of the regional and seasonal distribution of nitrogen in woody plants are apparently fragmentary and rare. Proebsting (1925) analyzed total nitrogen in the cortex-phloem and xylem of apple twigs from April to September in connection with his work on the relation of stored food to cambial activity in the apple. The work reported in this section is concerned with the distribution of the total organic and amino nitrogen fractions in the four complex tissues—periderm, cortex-phloem, outer xylem, and inner xylem-pith—of the 2-3 year portions of apple twigs throughout the year. The data are recorded in Tables X, XI, XII, and XIII, and are shown graphically in Figures 14 and 15.

Total Organic Nitrogen

The most striking fact brought out in Table X and the lower graph of Figure 14 is the extremely small amount of organic nitrogen in 2-3 year portions of apple twigs.

Table X shows that the organic nitrogen content of the complex tissues rarely rises above one per cent of the dry weight in the periderm and cortex-phloem and remains below 0.5 per cent at all times in the outer xylem and inner xylem-pith.

The lower graph of Figure 14 shows plainly that the periderm, cortex-phloem, outer xylem, and inner xylem-pith follow the same trend in organic nitrogen content. There are, however, differences in amount. The organic nitrogen content of the periderm and cortex-

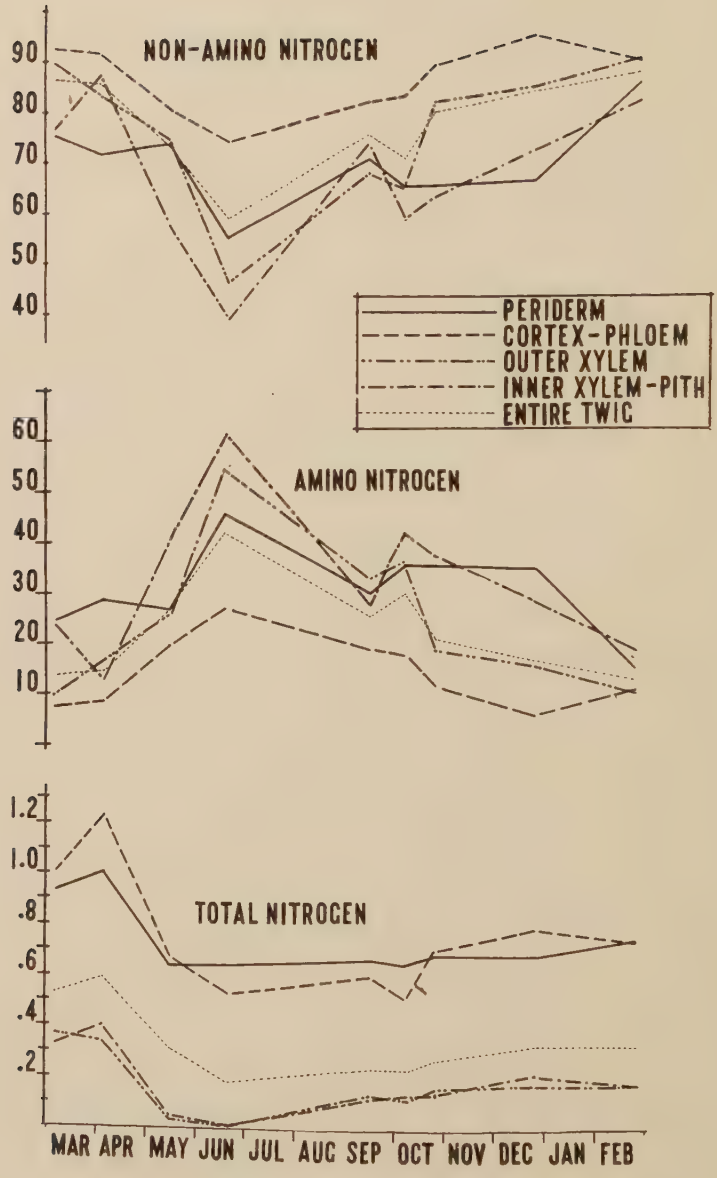

Fig. 14. Lower, seasonal distribution of total organic nitrogen in periderm, cortex-phloem, outer xylem, inner xylem-pith, and entire twig. Expressed in per cent dry weight. Upper, amino and non-amino nitrogen in the same complex tissues and the entire twig. Expressed in per cent of total organic nitrogen.

phloem is relatively much greater on a dry weight basis than that of the xylem tissues.

TABLE X

REGIONAL AND SEASONAL DISTRIBUTION OF TOTAL NITROGEN IN 2-3 YEAR PORTIONS OF APPLE TWIGS, MARCH 7, 1925, TO FEBRUARY 26, 1926

Expressed in per Cent Dry Weight

Date	Periderm	Cortex-phloem	Outer xylem	Inner xylem-pith	Entire twig
March 7	0.93	1.15	0.37	0.33	0.53
April 4	1.04	1.27	.35	.41	.59
May 16	0.63	0.67	.18	.20	.31
June 20	.63	.52	.07	.05	.17
September 17	.65	.59	.11	.13	.22
October 10	.63	.45	.13	.11	.22
October 23	.67	.69	.15	.17	.26
December 23	.67	.78	.20	.18	.32
February 26	0.74	0.73	0.18	0.18	0.32

The maximum in organic nitrogen reserves is reached in all the complex tissues during March-April. This maximum is no doubt partly due to the relative decrease in carbohydrates in the cortex-phloem. In the other tissues there is no decrease of carbohydrates but rather an increase in March-April.

The organic nitrogen reserves are at the highest just preceding the initiation of new growth. Rippel (1923) has reported experiments with *Sambucus nigra* showing that during the first spring growth translocation of nitrogen from the stem to the young shoots and leaves is very marked. A similar translocation from the apple twig would account for the rapid decline of organic nitrogen in spite of the fact that the total reserve of carbohydrates also shows a rapid decrease from April to May in some of the complex tissues.

During the summer both the organic nitrogen and the total reserve carbohydrate content are relatively constant, and there is only a minor drop at the beginning of October, when the total reserve carbohydrates increase very rapidly. In the dormant period during autumnal yellowing of the leaves, there is again a slight rise in total nitrogen until the end of December. That there is a migration of nitrogenous compounds from the leaves to the stem during autumnal yellowing has been demonstrated by Combes (1925), who made nitrogen determinations for the leaves and parts of the stem of green and yellowed specimens. The stems of the detached branches showed a greater increase of nitrogen than those left on the tree, and the lower leaves lost more than the upper. On a quantitative basis, Combes (1924) has shown with *Tilia, Acer, Fagus, Castanea,* and *Aesculus* that from one-half to two-thirds of the nitrogenous compounds in green leaves passes into the twigs during the normal yellowing. He also reported that no appreciable part is lost by leaching (rains and dew).

In spite of the fact that a considerable amount of nitrogen passes from the leaves into the twigs, there is no great gain in nitrogen on a dry weight basis. This may be accounted for in great measure by the proportionate increase in carbohydrates during the same period. That the nitrogen content remains constant or makes a slight gain during this period is evidence that the relative gain in nitrogen is proportionate to the gain in carbohydrates. On the basis of the entire twig, the same tendencies are shown as indicated for the complex tissues, but in amount of organic nitrogen on a dry weight basis, an intermediate position between that of the xylem and the periderm-cortex-phloem is maintained.

Amino and Non-amino Nitrogen

The amino acid content of the 2-3 year portions of apple twigs is exceedingly low, as shown in Table XI.

TABLE XI

REGIONAL AND SEASONAL DISTRIBUTION OF AMINO NITROGEN IN 2-3 YEAR PORTIONS OF APPLE TWIGS, MARCH 7, 1925, TO FEBRUARY 26, 1926

Expressed in per Cent Dry Weight

Date	Periderm	Cortex-phloem	Outer xylem	Inner xylem-pith	Entire twig
March 7	0.230	0.090	0.038	0.076	0.074
April 4	.296	.111	.059	.053	.085
May 16	.168	.133	.046	.085	.083
June 20	.286	.136	.038	.030	.071
September 17	.194	.109	.036	.036	.055
October 10	.221	.077	.045	.045	.065
October 23	.234	.077	.028	.063	.053
December 23	.231	.043	.031	.051	.052
February 26	0.111	0.078	0.019	0.034	0.041

According to this table and Figure 15, the percentage of amino-nitrogen in the periderm and cortex-phloem is usually higher than that in the xylem tissues. The amino-nitrogen content of the entire twig on a dry weight basis follows the trend of the outer xylem, but the amount is usually greater except during the first half of the dormant period.

The amino and non-amino nitrogen has been expressed as per cent of total organic nitrogen in Tables XII and XIII, and the upper two graphs of Figure 14.

These tables and the upper two graphs of Figure 14 show that amino nitrogen is relatively more abundant during the period of most rapid growth, May to July, and that non-amino nitrogen is relatively low during the same period. The non-amino nitrogen, however, is relatively more abundant during the dormant season, October to April, and amino nitrogen is relatively low during the same period. This is in harmony with the microchemical tests made by Kern (1923),

who reports that protein substances are much more plentiful in winter in the cortex-phloem and parenchyma than during the summer season.

TABLE XII

REGIONAL AND SEASONAL DISTRIBUTION OF AMINO NITROGEN IN 2-3 YEAR PORTIONS OF APPLE TWIGS, MARCH 7, 1925, TO FEBRUARY 26, 1926

Expressed in per Cent of Total Nitrogen

Date	Periderm	Cortex-phloem	Outer xylem	Inner xylem-pith	Entire twig
March 7	24.73	7.86	10.27	23.03	13.96
April 4	28.46	8.76	16.86	12.92	14.40
May 16	26.66	19.85	25.72	42.50	26.77
June 20	45.40	26.24	54.28	61.43	41.76
September 17	29.92	18.47	32.72	27.69	25.00
October 10	35.08	17.22	35.38	41.06	29.54
October 23	35.04	11.16	18.33	37.06	20.38
December 23	34.47	5.51	15.50	28.33	16.25
February 26	15.00	10.68	10.55	18.88	12.81

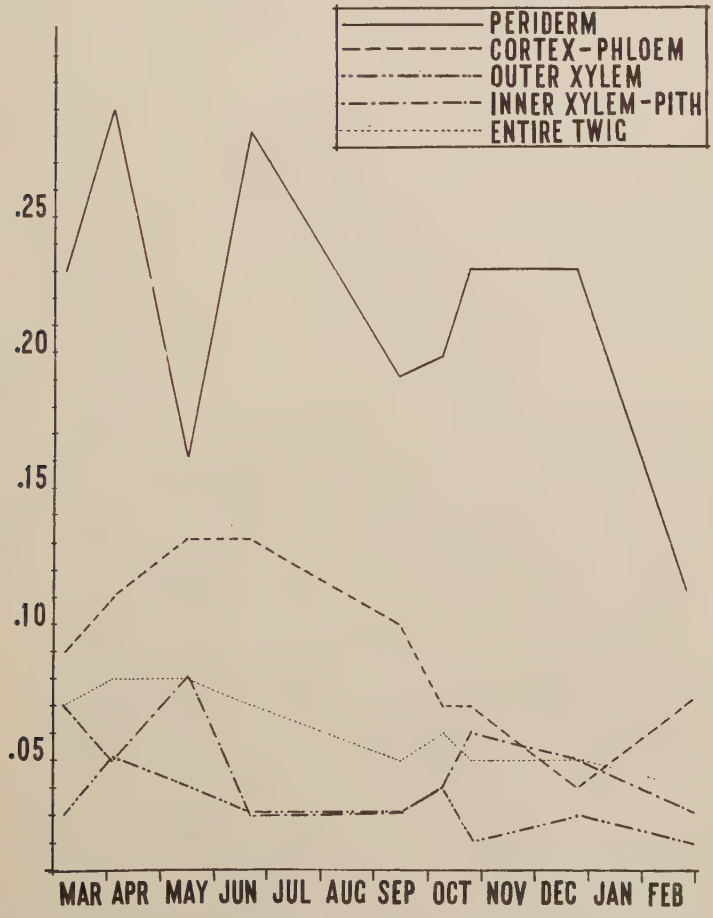

Fig. 15. Seasonal distribution of amino nitrogen in periderm, cortex-phloem, outer xylem, and inner xylem-pith. Expressed in per cent dry weight.

TABLE XIII

REGIONAL AND SEASONAL DISTRIBUTION OF NON-AMINO NITROGEN IN 2-3 YEAR PORTIONS OF APPLE TWIGS, MARCH 7, 1925, TO FEBRUARY 26, 1926

Expressed in per Cent of Total Nitrogen

Date	Periderm	Cortex-phloem	Outer xylem	Inner xylem-pith	Entire twig
March 7	75.27	92.14	89.73	76.97	86.04
April 4	71.54	91.24	83.14	87.08	85.60
May 16	73.34	80.15	74.28	57.50	73.23
June 20	54.60	73.76	45.72	38.57	58.24
September 17	70.08	81.53	67.28	72.31	75.00
October 10	64.92	82.78	64.62	58.94	70.46
October 23	64.96	88.84	81.67	62.94	79.62
December 23	65.53	94.49	84.50	71.67	83.75
February 26	85.00	89.32	89.45	81.12	87.19

REGIONAL AND SEASONAL DISTRIBUTION OF ASH

The apparent seasonal variation of ash in the complex tissues of the 2-3 year portions of apple twigs, is indicated in Table XIV and Figure 16.

TABLE XIV

REGIONAL AND SEASONAL DISTRIBUTION OF ASH IN 2-3 YEAR PORTIONS OF APPLE TWIGS, MARCH 7, 1925, TO FEBRUARY 26, 1926

Expressed in per Cent Dry Weight

Date	Periderm	Cortex-phloem	Outer xylem	Inner xylem-pith	Entire twig
March 7	2.51	6.88	0.61	0.60	1.84
		March 27*			
April 4	2.73	7.28	.61	.57	2.03
	April 18*				
May 16	2.53	7.67	.60	.51	2.13
	May 30*				
June 20	3.88	8.21	.62	.43	1.98
September 17	3.31	8.44	.49	.52	2.03
October 10	3.48	8.22	.59	.51	2.18
			October 4*		
October 23	3.42	6.46	.68	.69	1.73
	November 6*				
December 23	2.75	6.94	.66	.66	1.90
February 26	2.73	7.00	0.65	0.62	1.05

* Samples lost during ash analysis; figures for nearest available samples substituted with date indicated below.

The most striking fact brought out in Table XIV is the considerable difference in total ash content in periderm and cortex-phloem as contrasted with the xylem tissues. The cortex-phloem has the highest percentage, 6.46 to 8.44, followed by the periderm, 2.51 to 3.88. The woody tissues show only very slight variation. The total amount in each case is less than 1 per cent, 0.49 to 0.68 per cent (outer xylem) and 0.43 to 0.69 per cent (inner xylem-pith). The marked fluctuations in amount of ash in the cortex-phloem and periderm are probably only apparent. The curve for total carbohydrates in the case

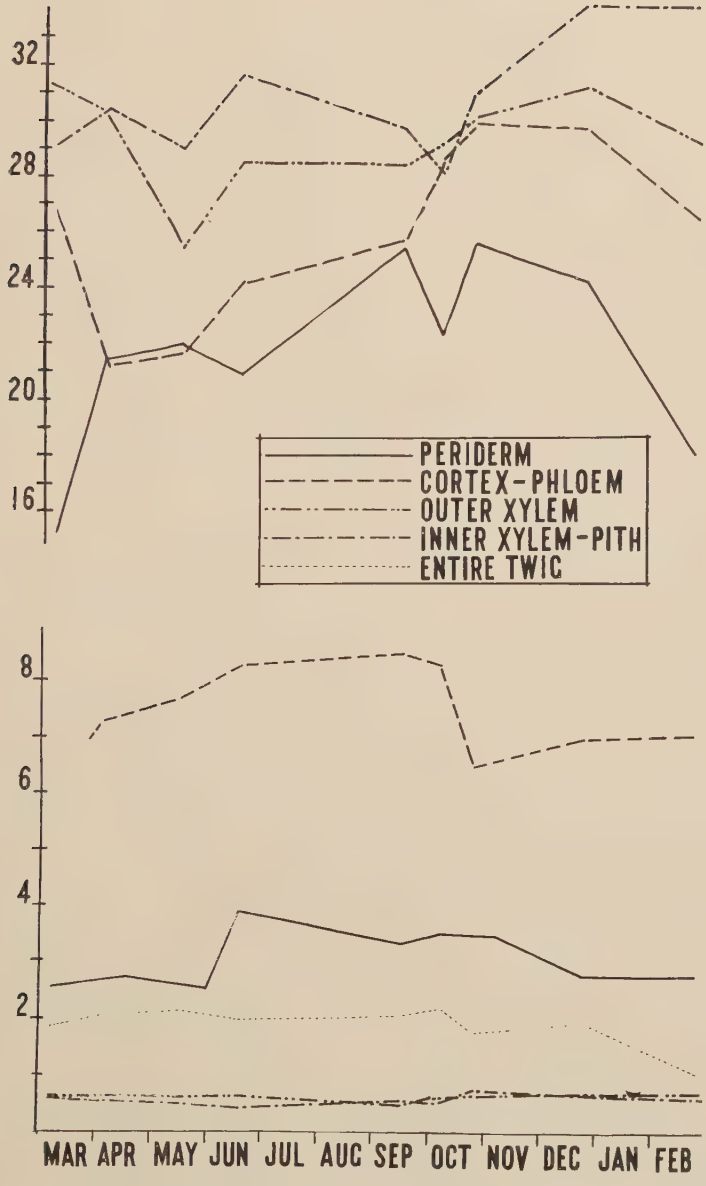

Fig. 16. Lower, seasonal and regional distribution of ash in periderm, cortex-phloem, outer xylem, inner xylem-pith, and entire twig. Expressed in per cent dry weight.

Upper, seasonal and regional distribution of total carbohydrates (except cellulose) in the four complex tissues—periderm, cortex-phloem, outer xylem, and inner xylem-pith. Expressed in per cent dry weight.

of the cortex-phloem is in some particulars the reverse of that for total ash; when the carbohydrates are relatively low during the growing season the ash content is highest, and when the reserve carbohydrates are translocated from the leaves to the twig at the time of leaf-fall, the ash is lowest. The same is, in general, true of the periderm. The fact that during the growing season both the ash and total reserve carbohydrates in the cortex-phloem show the same tendency indicates that the mineral matter is increased in proportion to the carbohydrates. The apparent decline of ash in the autumn is presumably the result of the relatively greater increase of total reserve carbohydrates, as shown in Figure 16. The ash content of the woody tissues is apparently quite constant, indicating that the ash constituents are increased in the same proportion as the carbohydrates.

When the ash content is expressed on the basis of the whole twig, the curve follows, in some particulars, that of the cortex-phloem, on a lesser scale.

Dormant Season

At the time of autumnal yellowing, part of the mineral constituents of the leaves is translocated to the stem. As early as 1878, Schröder demonstrated that potassium and phosphorus move back into the stem during the drying out of the leaves after freezing. Tucker and Tollens (1900) reported that the mineral elements in the leaves do not all pass to the twigs before leaf-fall, for rains and dews leach out a part.

Ramann (1912) pointed out that in frozen leaves the translocation of mineral constituents must take place in a short time between the thawing and drying of the frozen leaves. According to this authority, this may take place in a few hours.

The increase in mineral constituents in the twig due to the translocation of part of these elements from the leaves at the time of leaf-fall, is counterbalanced by the relatively great increase in total carbohydrates in the twig, so that in spite of the increase the percentage of ash on a dry weight basis shows an apparent decrease due to the relatively much greater proportion of carbohydrates. This is shown plainly for the periderm and cortex-phloem in Figure 16.

Migration of Mineral Constituents from Stem to Shoots and Leaves

The greater portion of the reserve mineral elements which have passed into the stem during normal autumnal yellowing is probably transported back to the expanding shoots in the spring together with mineral elements absorbed from the soil. Rippel (1923) reports that during the first spring growth of *Sambucus nigra* translocation of

potassium and phosphorus from the stem to the young shoots and leaves is very marked.

PRACTICAL APPLICATIONS

Altho the primary aim of the research was to add to our scientific knowledge of apple physiology, the work was undertaken with a realization of the practical bearing of such contributions on horticultural research and practice. A better understanding of the functioning of the complex tissues of the apple twig is of immediate interest in the study of such problems as (1) the technic of sampling twigs, roots, etc.; (2) nutrition, (3) winter hardiness, and (4) translocation.

Analysis of Plant Organs

In the discussion of methods of procedure it has already been pointed out that the variation in the proportion of the various complex tissues during the season, due primarily to the difference in diameter of the organ, to changes of weight independent of volume, and to growth and senescence of tissues, puts the gross analysis of plant organs, twigs, spurs, roots, etc., on the defensive. The error from these sources should be fully realized when seasonal studies of plant organs are undertaken.

Nutrition

Since the appearance of the work of Kraus and Kraybill (1918) on the carbohydrate-nitrogen ratio as related to fruit bud formation in the tomato, it has been customary to extend this concept to apple physiology. The ratio has been variously expressed by different workers—sugar, starch, or total reserve carbohydrates over nitrogen. In order to test the validity of such a procedure with 2-3 year portions of apple twigs, the data have been expressed in these terms for the entire twig and for the four complex tissues, periderm, cortex-phloem, outer xylem, and inner xylem-pith, as shown in Table XV. The ratios are shown graphically in Figures 17, 18, 19, and 20.

In Figure 17 the ratios are shown on the basis of the entire twig. The acid-hydrolyzable hexosan–nitrogen, and the total carbohydrate–nitrogen ratios follow the same tendencies, but the latter is very much higher at all seasons of the year. The total sugar–nitrogen ratio follows a trend different from the preceding ratios from September to February.

Figure 18 shows that the total sugar–nitrogen ratio for all the four complex tissues is similar, with differences in magnitude during certain periods in the year.

The hydrolyzable hexosan–nitrogen ratio shows differences in trend for the woody tissues as contrasted with the periderm and cortex-phloem. Figure 19 shows that in the xylem tissues the decline from

the summer maximum begins in June, but in the periderm and cortex-phloem there is a steady rise until September or October before a decline sets in.

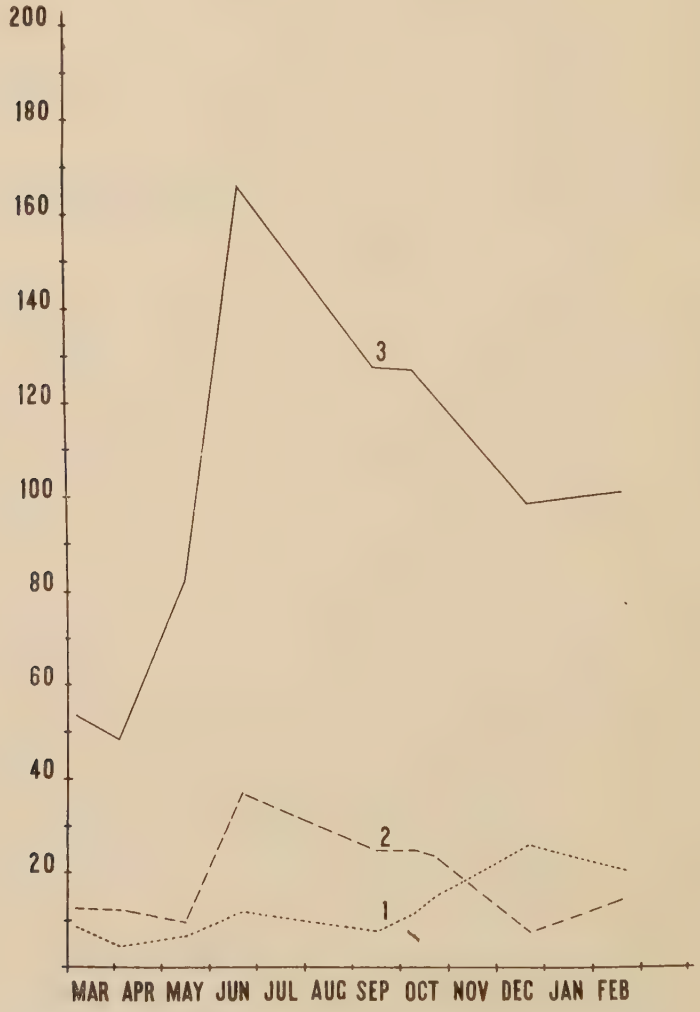

Fig. 17. Carbohydrate-nitrogen ratios; entire twig; total sugar-nitrogen ratio (1); acid-hydrolyzable hexosan-nitrogen ratio (2); and total carbohydrate-nitrogen ratio (3).

As shown in Figure 20, the total carbohydrate–nitrogen ratios show tendencies similar to those just described, but the ratios for the woody tissues are extremely high.

TABLE XV

CARBOHYDRATE-NITROGEN RATIOS: PERIDERM; CORTEX-PHLOEM; OUTER XYLEM; INNER XYLEM-PITH, AND WHOLE TWIGS; IN 2-3 YEAR PORTIONS OF APPLE TWIGS

MARCH 7, 1925, TO FEBRUARY 26, 1926

Date	Periderm	Cortex-phloem	Outer xylem	Inner xylem-pith	Entire twig
		Total Carbohydrates / Total Nitrogen			
March 7	16.24	23.21	84.02	88.00	53.56
April 4	20.58	16.76	86.71	78.92	48.28
May 16	34.79	32.19	141.33	144.95	81.80
June 20	33.22	46.32	407.14	632.80	165.70
September 17	39.07	49.34	257.45	222.07	126.77
October 10	35.26	59.26	223.30	256.00	126.09
October 23	38.07	43.33	200.33	181.41	119.76
December 23	36.94	38.05	155.45	183.94	97.31
February 26	24.17	36.09	159.16	183.83	91.75
		Total Sugars / Total Nitrogen			
March 7	4.72	8.35	9.00	9.78	8.49
April 4	5.76	4.33	3.42	3.34	4.15
May 16	10.14	6.14	4.94	5.30	6.15
June 20	9.41	10.78	11.57	16.60	11.58
September 17	7.53	9.25	5.18	5.46	7.59
October 10	9.31	12.57	7.00	8.72	11.40
October 23	14.67	17.49	11.60	13.35	14.96
December 23	17.97	16.88	29.30	45.05	25.96
February 26	8.60	12.15	23.82	34.22	20.28
		Acid-Hydrolyzable Hexosans / Total Nitrogen			
March 7	1.27	2.80	19.64	27.27	12.45
April 4	2.49	3.73	20.22	25.09	12.23
May 16	1.65	4.04	5.50	33.80	9.48
June 20	7.90	8.61	81.28	165.80	36.82
September 17	11.32	10.47	44.00	40.23	24.13
October 10	8.96	15.73	44.38	35.90	24.36
October 23	2.22	1.88	46.73	45.70	23.61
December 23	0.35	0.88	15.65	17.55	7.90
February 26	2.06	3.23	25.11	28.11	14.18

In all the cases considered above, the ratios for the entire twig follow the trend of the woody tissues as shown in Figures 18, 19, and 20. Apparently the results obtained by analyzing entire twigs would give values in most cases that approach the tendencies inherent in the xylem, the moisture transport, and the storage organ, since the trend in the cortex-phloem is not the same except in the total sugar–nitrogen ratios.

Winter Hardiness

Experiments by Beaumont and Hildreth (1927) and other workers have shown that there is a difference in the resistance of the various tissues of the terminal apple twig to low temperatures during the winter. In such researches the microchemical observations should be reinforced by macrochemical analyses whenever possible. By studying the complex tissues separately, a basis would be afforded for in-

terpreting, if possible, the variability to cold resistance on a quantitative chemical basis.

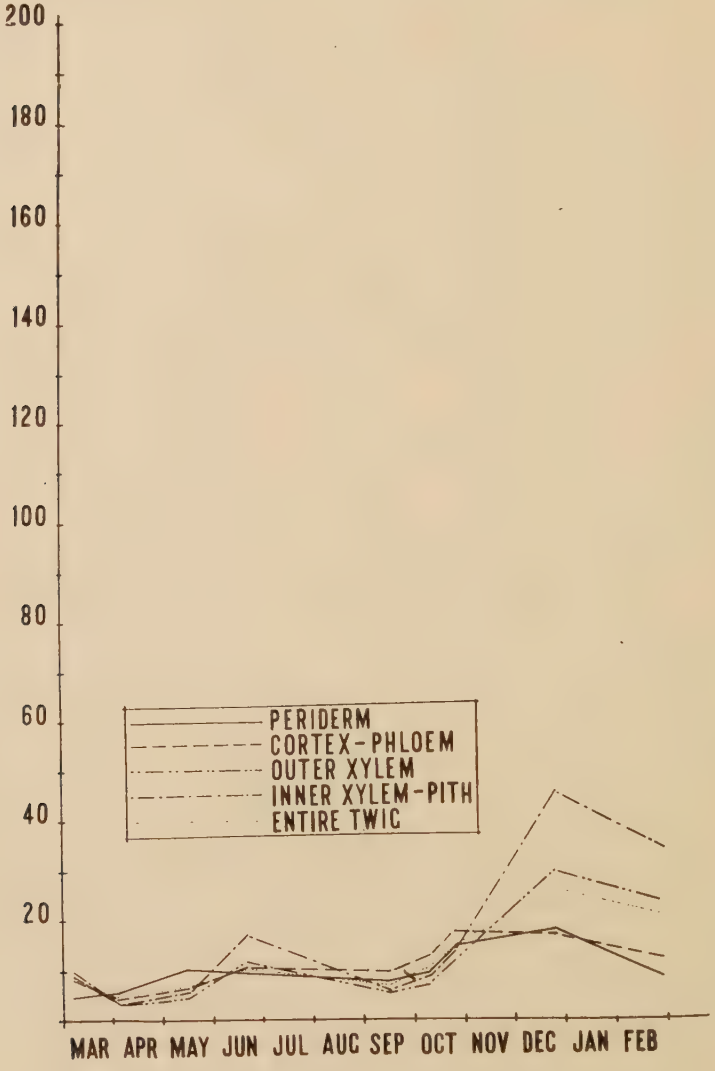

Fig. 18. Total sugar-nitrogen ratios; periderm, cortex-phloem, outer xylem, inner xylem-pith, and entire twig.

Translocation

In order to obtain an adequate conception of the translocation of materials in the tree as a basis for nutritional studies, the methods described should be extended to the entire tree—roots, trunk, branches, twigs, first-year shoots, buds, leaves, flowers, and fruits. The extent

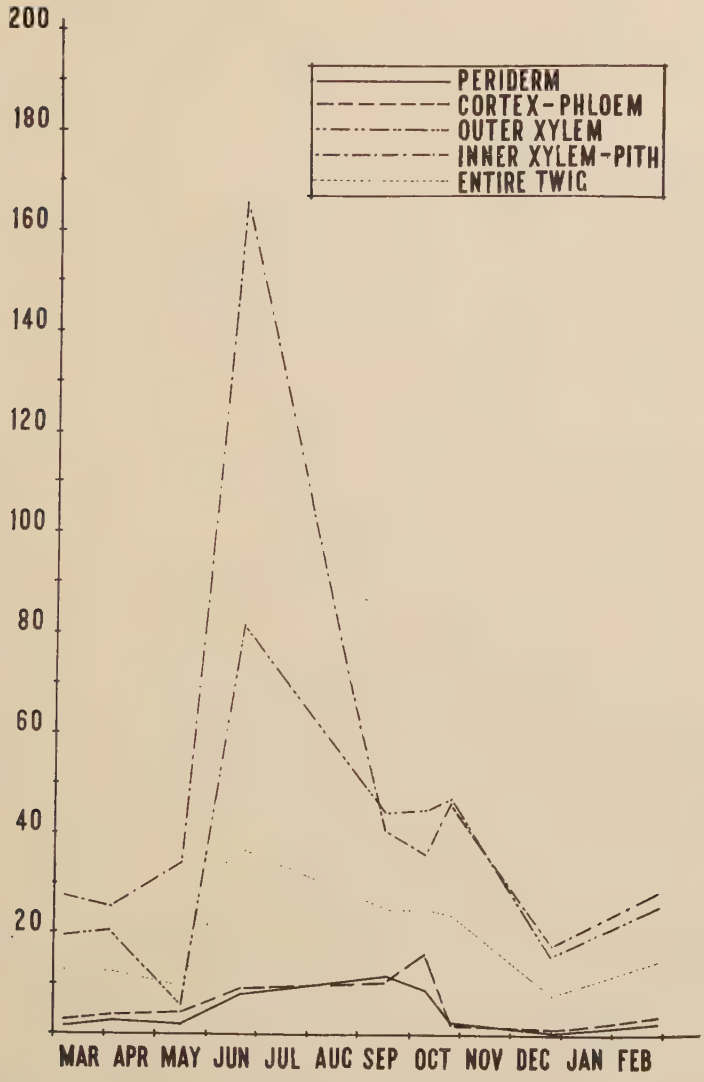

Fig. 19. Acid-hydrolyzable hexosan-nitrogen ratios; periderm, cortex-phloem, outer xylem, inner xylem-pith, and entire twig.

of the separation of the complex tissues would depend upon the nature of the organ, but the regional study should be made whenever practicable. For such a survey, 6-8 year trees, on their own roots, growing under the same conditions and with similar performance records, should be utilized. If possible, the experiments should be carried out on trees grown in tubs in the greenhouse under controlled temperature and moisture conditions. The data, when interpreted, would afford

a much truer conception of the translocation of materials on a quantitative basis, and would reveal a dynamic picture of the functioning of the tree as a whole.

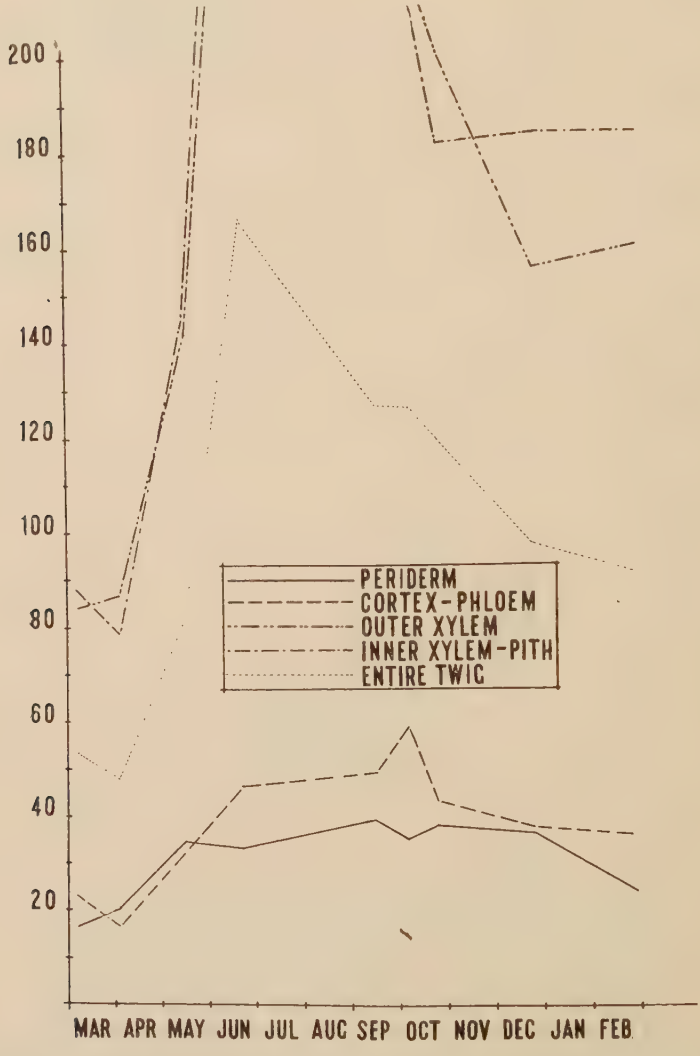

Fig. 20. Total carbohydrate-nitrogen ratios; periderm, cortex-phloem, outer xylem, inner xylem-pith, and entire twig.

CONCLUSIONS

The results presented and discussed in the preceding pages may now be summarized.

Methods

1. A method has been suggested for the separation of the complex tissues of woody plant stems for macrochemical analysis.

2. The macrochemical analysis of plant organs in seasonal studies has been put on the defensive. The error introduced by the difference in diameter of twigs and seasonal changes in absolute amount of dry matter independent of volume in the complex tissues, may be quite significant.

Moisture

3. In 2-3 year portions of apple twigs, the moisture content of the cortex-phloem is always highest and that of the periderm usually lowest. The two complex tissues, outer xylem and inner xylem-pith, follow the same general trend except that the percentage of moisture in the outer xylem shows a tendency to run higher at practically all times of the year. This is especially true during summer.

4. The summer moisture maximum in all the tissues was reached during the season of greatest precipitation, over 70 per cent.

5. The outer xylem apparently serves as the complex water-supplying tissue. The moisture content of this complex tissue follows the rainfall curve fairly closely. The complex tissues—periderm and cortex-phloem—seem to act independently of the total precipitation after a certain maximum has been reached, selecting the amount of water required from the common supplying tissues.

6. The drop in moisture of the periderm in June may be due to the differentiation of the phellem—with the cessation of life processes the moisture relations are those of dead suberized tissue.

7. On the basis of the entire twig, the individuality of the complex tissues naturally disappears and the tendency of the tissue in greatest relative abundance, outer xylem, is reflected.

8. The temporary decline in moisture content during the transition periods, March to April, and September to October, can not be adequately explained from the present data. It appears, however, that the moisture decline may be bound up with the chemical and physical changes of the protoplasm during the transition periods.

9. During the first part of the dormant period, October to December or January, there is a fairly marked decline in moisture content of all tissues. After this time until March, the moisture rises rapidly in the cortex-phloem and slightly in the inner xylem-pith, and declines slightly in the outer xylem. There is a continual decline in moisture content in the periderm during the entire period.

10. The trend in moisture content of the cortex-phloem and woody tissues is similar throughout the year, but the cortex-phloem is much more subject to marked fluctuations within the general trend, the outer xylem less so, and the inner xylem-pith least of all. The trend of the moisture content of the periderm departs from that of the other tissues in June to July.

11. From the end of December to the end of January, the moisture content of the complex tissues is not constant, but when the data are expressed on the basis of the entire twig the moisture content is practically constant.

Carbohydrates

12. On a dry weight basis, the total reserve carbohydrate content of the woody tissues is higher than that of the cortex-phloem at all times of the year, and that of the periderm is usually lower than that of the cortex-phloem.

13. The maximum in total reserve carbohydrates is reached in all the tissues after leaf-fall.

14. The pentosan content forms a relatively large proportion of the total reserve carbohydrates.

15. The fluctuations in pentosans at the beginning of the growing season are in great measure due to relative decreases in acid-hydrolyzable hexosans. Increases on a dry weight basis after leaf-fall disappear when expressed on the basis of per cent total reserve carbohydrates, showing that the pentosan content rises in proportion to the other reserve carbohydrates in the periderm and cortex-phloem. In the xylem tissues the increase in pentosans is relatively less than that of other reserve carbohydrates.

16. The acid-hydrolyzable hexosan content of the xylem tissues is usually greater than that of the periderm and cortex-phloem, and the latter reach a lower minimum than the former.

17. During the summer the acid-hydrolyzable hexosan content is built up in the periderm, cortex-phloem, and xylem tissues. In the xylem tissues there is a decline to a minimum in early October with a quick rise during the same month.

18. With the approach of cold weather there is a quantitative change of hexosans into sugar, beginning in September-October for the periderm and cortex-phloem and in October for the xylem tissues. The total sugars are also increased, owing to translocation from the leaves at time of autumnal yellowing.

19. There is a general depletion of hexosans in May in all the tissues, owing to translocation of sugar used in growth extension.

20. In the periderm and cortex-phloem there are two hexosan maxima, September-October and March-April, and two hexosan minima,

December-January and May. In the xylem tissues there are apparently three hexosan maxima, October-November, March-April, and June; and three hexosan minima, September-October, December-January, and May.

21. During the winter minimum the hexosans disappear almost completely in the periderm and cortex-phloem, and are reduced more than 50 per cent in the xylem tissues. The value for sugars is twice as great as that of the hexosans at the minimum. This is not in agreement with the work of Alfred Fischer (1891). The difference in results is due to the technic used by Fischer, which gave him only a measure of the glucose in the vessels of the xylem.

22. The total sugar content of the periderm and cortex-phloem is always markedly higher than that of the xylem tissues.

23. All the tissues follow the same trend as far as total sugars are concerned.

24. During the winter the reducing sugar content of the periderm and cortex-phloem is higher than that of the non-reducing sugars, but this is reversed in the xylem tissues.

Nitrogen

25. The nitrogen content of the complex tissues of 2-3 year portions of apple twigs is extremely low, rarely rising above one per cent on a dry-weight basis in the periderm and cortex-phloem, and remaining below 0.5 per cent at all times in the outer xylem and inner xylem-pith.

26. As regards nitrogen content, the periderm, cortex-phloem, outer xylem, and inner xylem-pith follow the same trend throughout the year.

27. The nitrogen maximum is reached in March-April just preceding rapid growth extension.

28. After the marked decline in nitrogen with rapid growth extension, the nitrogen content is relatively constant during the summer.

29. From leaf-fall and through the winter the nitrogen content remains practically constant, with very slight downward or upward changes. This means that the nitrogen content increases in proportion to the carbohydrates in the fall, and the slight changes may be due to loss of combustible material by respiration or gain or loss in dry matter by translocation.

30. The amino-nitrogen content is highest during the growing season, decreases as the rest period approaches, and reaches a minimum during the dormant season.

31. The non-amino or protein nitrogen is highest during the dormant season, decreases as the active growing season approaches, and reaches a minimum in June.

32. The non-amino or protein nitrogen is consistently higher in the cortex-phloem than in the periderm and xylem tissues.

Ash

33. The amount of ash in the periderm, 2.51 to 3.48 per cent on a dry weight basis; cortex-phloem, 6.46 to 8.44 per cent, is higher than the mineral constituents in the xylem tissues, always below 0.7 per cent.

34. The fluctuations in ash content in the periderm and cortex-phloem may be misleading, and are apparently due to the relatively great fluctuations in total reserve carbohydrates. An apparent decline is indicated at the time a portion of the mineral constituents of the leaves is transported to the stem. This apparent decline is the result of the relatively greater increase of total reserve carbohydrates.

35. The ash content of the woody tissues is relatively constant, showing that the ash constituents are increased in proportion to the carbohydrates.

36. The increase of ash in cortex-phloem during the growing season shows the same tendency as the rise in total reserve carbohydrates, indicating that the mineral constituents are increased in proportion to the carbohydrates during this period.

SUMMARY

A method of separating the complex tissues of 2-3 year portions of apple twigs, periderm, cortex-phloem, outer-xylem, and inner xylem-pith, for the purpose of macrochemical analysis is described. The method may be applied to other plants.

The variation in the relative proportions of the different complex tissues during the season, owing to the difference in the diameter of the organ, to changes of weight independent of volume, and to the growth and senescence of tissues, points to the fact that in the gross analysis of plant organs—twigs, spurs, roots, etc.—in seasonal physiological studies, errors may be introduced which may minimize the usefulness of the results.

The seasonal macrochemical analysis of the four complex tissues of 2-3 year portions of apple twigs for moisture, carbohydrate, nitrogen, and ash fractions shows that the gross macrochemical analysis of plant organs (entire twigs) does not give a true picture for physiological studies. In most cases, when the data are expressed on the basis of the entire twig the tendency of one of the complex tissues, outer xylem, in greatest relative abundance, is reflected overshadowing the individuality of the other complex tissues. Accordingly, in seasonal macrochemical physiological studies, the complex tissues of the entire organ should be separately analyzed.

The seasonal changes in the moisture, carbohydrate, nitrogen, and ash fractions of four complex tissues, periderm, cortex-phloem, outer xylem, and inner xylem-pith are traced and the rôle of such changes in the normal physiology of the woody stem is discussed.

The application of the method described in the study of 2-3 year portions of apple twigs under central Minnesota conditions seems to indicate that the conclusions of Alfred Fischer (1891) regarding the "Stärkebäume" (starch-storing trees), do not hold for the apple variety studied. The increase in total sugars and a decline of acid-hydrolyzable hexosans, in both the cortex-phloem and the xylem tissues, with the approach of winter is apparently the outstanding fact from the standpoint of food storage during the dormant season. Such trees should more properly be designated as "Zuckerbäume" (sugar-storing trees).

The usefulness of the method described in the study of translocation, nutrition, and winter hardiness in plants is discussed.

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